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14. ABSTRACT

The majority of patients with spinal cord injury (SCI) develop intractable chronic neuropathic pain that is resistant to conventional pharmacologic treatments. An alternative and potentially effective modality of treatment—motor cortex stimulation (MCS)—offers hope for these patients. The purpose of this application is to elucidate the neurobiological basis of reduced pain following MCS. We propose that MCS reverses hyperalgesia by enhancing the activity in the GABAergic nucleus zona incerta (ZI), and therefore inhibiting pain processing in the posterior thalamus (PO). Using single cell extracellular electrophysiological recordings from the thalamus of rats with SCI-pain we tested the effect of MCS on the activity of neurons in ZI and PO. MCS significantly enhanced spontaneous and evoked responses in the majority of ZI neurons and caused a significant and robust suppression of activity in PO. These findings are consistent with our overarching hypothesis that MCS alleviates pain by activating the incerto-thalamic pathway.

15. SUBJECT TERMS
Zona Incerta, Posterior Thalamus, Spinal Cord Injury, Pain, Electrical Stimulation

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INTRODUCTION

A majority of patients develop maladaptive plastic changes within the central nervous system following spinal cord injury. These changes result in abnormal regulation of peripheral inputs, and impaired perception of tactile and painful stimuli. The majority of these patients suffer because conventional treatment fails to reverse these maladaptive changes. An alternative and potentially effective modality of treatment—motor cortex stimulation (MCS)—offers hope for these patients. The goal of the experiments presented in this annual report is to elucidate the neurobiological basis of reduced pain following MCS. We propose that *MCS reverses hyperalgesia by enhancing the activity in the GABAergic nucleus zona incerta (ZI), and therefore inhibiting pain processing in the posterior thalamus (PO).*

In the first year of funding, we focused on completing the experiments in the first task (**Task 1**) as outlined in the Statement of Work. The experiments were fruitful and results exciting and the progress was well within the proposed time line.

Task 1 was to demonstrate that MCS enhances inhibitory inputs from the inhibitory nucleus ZI to the PO. In this task we proposed to complete 3 subtasks in the first year: **Task 1a.** was to secure approval on animal use and care. This task was completed and all procedures were reviewed and approved by a University of Maryland IACUC and the Department of Defense ACURO; **Task 1b**. was to illustrate that MCS enhances the activity of ZI neurons that project to PO and we completed this experiment in the proposed time (4-10 months); and **Task 1c.** was to illustrate that MCS suppresses evoked and spontaneous activity of PO neurons. We are on schedule to complete these experiments in the proposed time (8-14 months).

BODY

Task 1b

To illustrate that MCS enhances the activity of ZI neurons that project to PO *(4-10 months)*.

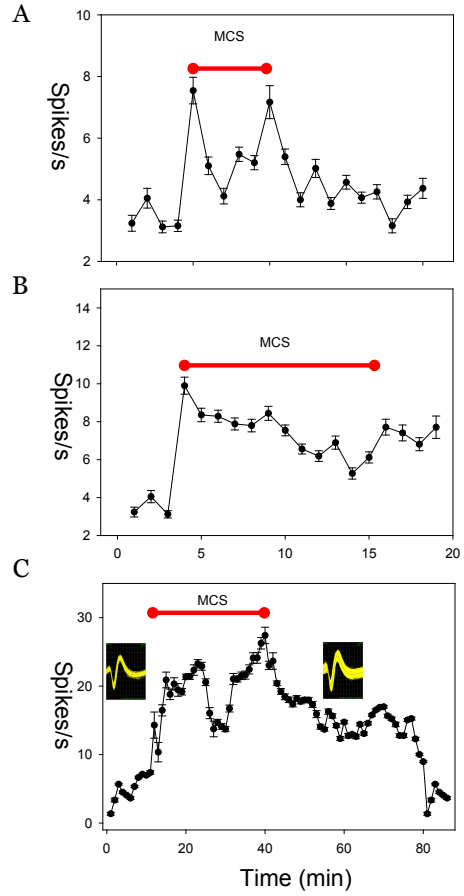

Figure 1. MCS enhances spontaneous activity in ZI neurons. (A) Responses of a representative ZI neuron to MCS are shown. MCS for 5 minutes (red bar) resulted in a short lasting enhancement of spontaneous firing rate immediately after motor cortex stimulation. (B) In another ZI neurons, electrical stimulation for 15 minutes results in a significant sustained increase in spontaneous firing rate immediately after MCS (the neuron was lost after 20 minutes of recording). (C) MCS for 30 minutes in this neuron resulted in a prolonged significant enhancement in ZI spontaneous activity. Spontaneous activity remained elevated for approximately 40 minutes after the end of stimulations before the mean firing rate returned to baseline values. Insets are the shape matching templates used to sort the neuron before and after MCS.

To perform these experiments we adopted a model of spinal cord injury (SCI) that recapitulates the clinical characteristics of SCI-pain (Wang and Thompson, 2008; Masri et al., 2009). In this model, we place unilateral electrolytic lesions in the anterolateral quadrant of the spinal cord at the level of C6-T2. These lesions result in diffuse, bilateral mechanical hyperalgesia in the hindpaws approximately 2-3 weeks after injury (see **Detailed Methods**). All the experiments were performed in animals with SCI that exhibited frank hyperalgesia.

In anesthetized animals with SCI-pain we recorded *in vivo* extracellular activity of well-isolated ZI neurons (see **Detailed Methods**) and assessed their responses to MCS. We stimulated the motor cortex at intensity: 50 µA, frequency: 50 Hz, pulse duration: 300 µs because we found these parameters to be most effective in reducing hyperalgesia and spontaneous pain in our animal model of SCI-pain (Lucas et al., 2011; Davoody et al., 2011; **Appendix**).

For each cell in ZI we recorded: (**1**) Spontaneous activity for at least 5 minutes; (**2**) Responses to noxious and innocuous mechanical stimulation of the receptive field; and (**3**) Spontaneous activity and evoked activity during and immediately after MCS and until the cell recovered. Although we recorded neuronal activity during MCS, we did not include this data in our analysis because the electrical stimulus artifact was large and masked neuronal activity in most instances.

For each individual neuron, the mean firing rate was calculated every minute before and after MCS. Changes in mean firing rate of spontaneous activity over time were assessed

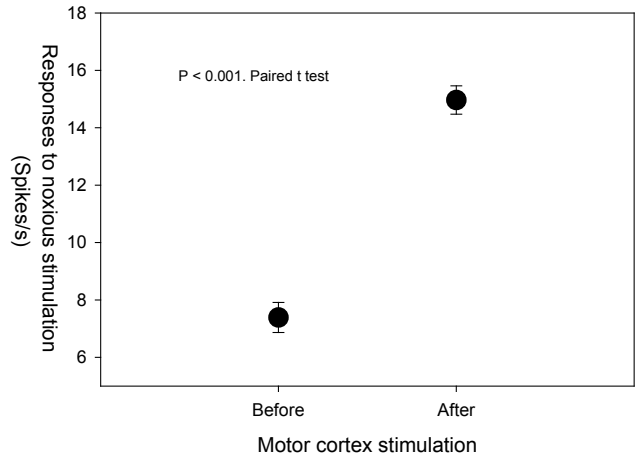

Figure 2. A representative example of a ZI neuron in response to application of electronic von-frey (200 gm,10 times) before and immediately after MCS. MCS was performed for 30 minutes. The evoked responses of this neuron were significantly enhanced.

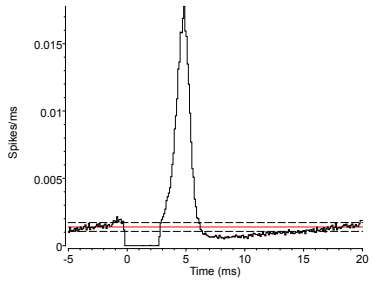

Figure3. Peristimulus histogram (Bin=1ms) is constructed from responses of a single ZI neuron to electrical stimulation of the motor cortex. Horizontal lines represent the mean (red) and the 99% confidence interval (black, dashed).

using Repeated Measures ANOVA when the data was normally distributed and Repeated Measures ANOVA on Ranks when the data was not. To assess changes in evoked activity before and after MCS, a paired "t" test or a Mann Whitney U test were used depending on the distribution of the data. A $p < 0.05$ was considered significant.

We recorded from 29 well-isolated ZI cells (the location of all neurons was confirmed using post-mortem histological analysis). In 38% (11/29) of the neurons, the activity of ZI was enhanced following MCS (Range: 8%-260% enhanced activity; $P<0.05$). Spontaneous activity was suppressed in only 7% (2/29) of ZI cells after MCS and the remaining cells were not affected (examples of ZI responses are shown in Fig. 1). As expected, the enhanced spontaneous activity of ZI after MCS in all of the 11 neurons was associated with enhanced responses to innocuous and noxious mechanical stimulation (see **Detailed Methods**). Figure 2 depicts a ZI neuron that responded to noxious mechanical stimulation (200 gm probe applied to the receptive field) before and after MCS. The mean firing rate increased from 7.4 ± 5.7 to 15.0 ± 5.4 spikes/s immediately after 30 minutes of MCS ($p<0.001$; Fig. 2). The same was true for the neurons that exhibited suppression in spontaneous activity after MCS (n=2) and in these neurons MCS suppressed the evoked responses.Taken together these findings illustrate that MCS results in enhanced activity in a large proportion of ZI neurons. They are consistent with our hypothesis that MCS produces analgesia by activating the incerto-thalamic pathway.

One important finding was that the magnitude and duration of enhanced activity in ZI neurons were dependent on the duration of MCS (Fig. 1). Electrical stimulation of the motor cortex for 5 minutes resulted in enhanced ZI activity that lasted for short periods of time (range: 1-5 min, $p<0.002$). Longer periods of stimulation (15 or 30 minutes) produced a significant enhancement in ZI activity that lasted for prolonged periods of time (range: 5-42 min, $p<0.004$). These observations are consistent with our recent findings that duration of analgesia produced after MCS is dependent upon the duration of electrical stimulation

(Lucas et al., 2011; **Appendix**).

Characteristics of ZI neurons enhanced by MCS

We specifically targeted our recordings to the ventral portions of ZI (ZIv), because we have shown previously that the highest density of GABAergic PO-projecting cells is located there (Trageser et al., 2006). It is also the area that receives the densest inputs from M1 (Urbain and Deschenes, 2007). We characterized ZI neurons by performing: (**1**) Orthodromic electrical stimulation of the motor cortex and; (**2**) Antidromic electrical stimulation of PO. Of the ZI neurons that were enhanced by MCS, a large proportion (82%) responded robustly and reliably with short latency (5-6 ms) to orthodromic stimulation of the motor cortex (e.g.: Fig. 3). These findings suggest that these neurons receive direct inputs from the motor cortex. For the anitdromic stimulation experiments, the yield was disappointingly low and we were able to perform antidromic stimulation successfully in only 1 neuron out of all ZI neurons that were enhanced by MCS.

Despite the low yield of the antidromic stimulation experiments, **Task 1b** was completed in the anticipated time (4- 10 months) and the findings are consistent with our overarching hypothesis that MCS reduces hyperalgesia by activating the incerto-thalamic pathway.

Task 1c

To illustrate that MCS suppresses evoked and spontaneous activity of PO neurons *(8-14 months)*.

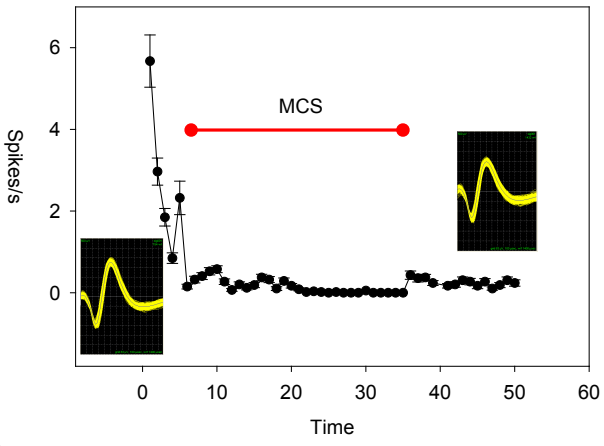

Figure 4. A representative example of PO responses to MCS is shown. In this neuron, the motor cortex was stimulated for 30 min. MCS (red bar) resulted in a robust suppression in spontaneous activity that lasted for the duration of recording.

In this experiment, we used similar methods to those described in **Task 1b** and utilized animals with SCI-pain. We targeted PO and recorded from 20 well-isolated neurons the location of which was confirmed using postmortem histological analysis. We obtained the same electrophysiological metrics described above and performed similar statistical analysis on each individual neuron.

In 75% (15/20) of the neurons, the activity of PO was suppressed significantly following MCS (Range: -10% to -97% suppressed activity; $P<0.001$). Spontaneous activity was enhanced in only 10% (2/20) of PO cells after MCS and the remaining cells were not affected (e.g.: Fig. 4).

The suppressed spontaneous activity of PO after MCS in all of the 15 neurons was associated with suppressed responses to innocuous and noxious mechanical stimulation. Figure 5 depicts an example of a PO neuron that responded to innocuous mechanical stimulation (20 gm probe applied to the receptive field) before and after MCS. The mean

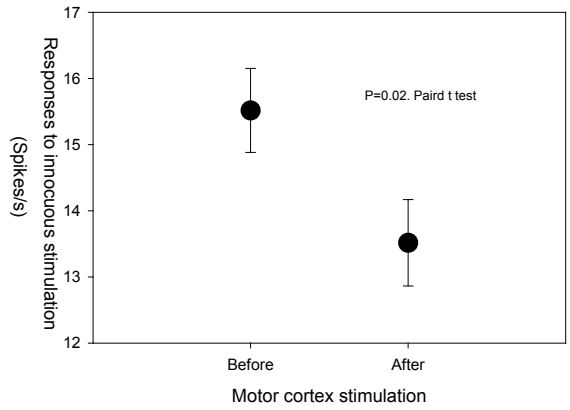

Figure 5. A representative example of a PO neuron in response to the application of electronic von-frey (20 gm, 10 times) before and immediately after MCS. MCS was performed for 30 minutes.

firing rate was suppressed from 15.5 ± 4.9 to 13.5 ± 5.1 spikes/s immediately after MCS (p=0.02; Fig. 5). The same was true for neurons that exhibited enhancement in spontaneous activity after MCS (n=2) and in these neurons MCS also enhanced the evoked responses.

These findings illustrate that MCS results in suppressed activity in the majority of PO neurons and they are consistent with our hypothesis that MCS produces analgesia by activating the incerto-thalamic pathway.

Similar to MCS effects on ZI, the magnitude and duration of suppressed activity in PO neurons was dependent on the duration of MCS. Electrical stimulation of the motor cortex for 5 minutes resulted in suppressed activity that lasted for short periods (range: 1-8 min, $p<0.002$). Longer periods of stimulation (15 or 30 minutes) produced a significant suppression in PO activity that lasted for prolonged periods (range: 20-50 min, $p<0.004$). These observations are consistent with our recent findings that duration of analgesia produced after MCS is dependent upon the duration of electrical stimulation (Lucas et al., 2011; **Appendix**).

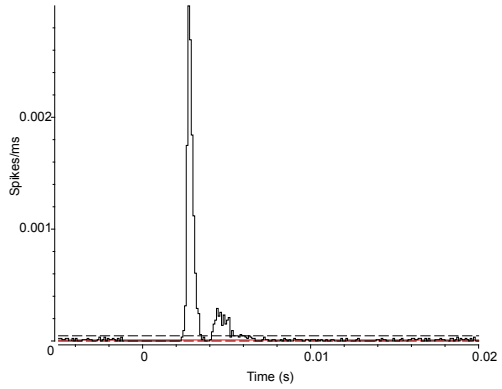

Figure 6. Peristimulus histogram (Bin=1ms) is constructed from responses of a single PO neuron to electrical stimulation of the motor cortex. Horizontal lines represent the mean (red) and the 99% confidence interval (black).

Characteristics of PO neurons enhanced by MCS

PO neurons were characterized by performing orthodromic electrical stimulation of the motor cortex. Of the 15 PO neurons that were suppressed by MCS, only 13% responded robustly and reliably with short latency (3 ms) to orthodromic stimulation of the motor cortex (e.g: Fig. 6). Contrary to ZI neurons, these findings suggest that a minority of PO neurons receive direct inputs from the motor cortex.

The proposed time line for **Task 1c** was (8-14 months) and although we collected enough data to reach solid conclusions (20 PO units), we will continue to increase our sample size and expect that in two months, data collection and analysis for this experiment will be complete. This is in agreement with the timeline proposed for this task in the Statement of Work.

Detailed Methods

Spinal cord lesion. Under aseptic conditions, and using ketamine/xylazine anesthesia

(80/10 mg/kg, i.m), a laminectomy to expose the spinal cord between C6 and T2 was performed and the dura was removed. A metal electrode (5 μm tip) was targeted to the anterolateral quadrant in one side of the spinal cord. Current (10 μA for 40 sec) was passed through the electrode to produce an electrolytic lesion. The muscles and skin were sutured in layers to approximate the incision sites. The location of the spinal lesions was assessed after the completion of the experiment in all the animals using postmortem histological analysis.

Behavioral testing. The animals were habituated for two weeks before behavioral testing. Mechanical and thermal hyperalgesia for animals was tested on three consecutive days before the spinal lesion surgery, at day 3 post-surgery, at day 7 post-surgery, and at weekly intervals thereafter as described in (Lucas et al., 2011; **Appendix**).

***In vivo* recording in anesthetized animals.** At least 21 days after spinal lesion surgery, animals with confirmed SCI-pain were anesthetized with an intra-peritoneal injection of urethane (1.5 g/kg). The bone overlying the contralateral M1 and the thalamus (in relation to the spinal lesion site) was removed. Extracellular unit recordings were performed using quartz-insulated tungsten electrodes (2 to 4 MΩ). The electrodes were advanced based on stereotaxic coordinates to target ZI, or PO.

Innocuous and Noxious Mechanical Stimulation. Electronic von frey was applied to the receptive field of the isolated neuron (ZI or PO) on the hindpaw or the face. A gradual force spanning both the innocuous and noxious range (6-300 gm) was applied. The application of mechanical stimuli was repeated 10 times before MCS and 10 times immediately after MCS.

Motor Cortex Stimulation. The hindpaw representation within M1 was identified using electrical microstimulation. Once the hindpaw representation is located, epidural bipolar insulated platinum electrodes (diameter: 70μm, exposed tip: 50μm, distance between the electrodes: 500μm) was secured using skull screws and cemented using dental resin. These electrodes were used for MCS and for orthodromic stimulation of ZI.

Antidromic stimulation. ZI-projecting PO neurons were identified by antidromic microstimulation with an electrode placed in PO. We recorded the latency of the antidromically evoked response (Swadlow, 1989) and performed the collision test (Bishop and King, 1982; Swadlow and Weyand, 1987). The yield was disappointingly low for this experiment because of the proximity between the recording and stimulation electrodes.

Histology. To identify recording sites in both acute and chronic recording experiments, at the end of the experiments, we made electrolytic lesions (5 μA for 10 sec) at, and then deeply anesthetized the rats with sodium pentobarbital (60 mg/kg). The rats were perfused transcardially with buffered saline followed by 4% buffered paraformaldehyde. We obtained coronal brain sections (70 μm thick) and Nissl-stained them. The sections were examined under the microscope to identify recording tracts, lesion sites stimulating electrodes location.

KEY RESEARCH ACCOMPLISHMENTS

The following is a list of the key research accomplishments during the first year of funding in this project:

- We obtained approval for animal care and use protocol from the University of Maryland Baltimore IACUC and ACURO (**Task 1a**).
- We illustrated that motor cortex stimulation (MCS) enhances spontaneous and evoked activity of a large proportion of zona incerta (ZI) neurons (**Task 1b**).
- We demonstrated that units enhanced by MCS are located in the ventral portion of zona incerta (ZIv)–the area where neurons that project to the posterior thalamus (PO) are localized (**Task 1b**).
- We found that the majority of ZI units enhanced by MCS receive direct inputs from the motor cortex (**Task 1b**).
- We demonstrated that MCS suppresses evoked and spontaneous activity in the majority of PO neurons (**Task 1c**).
- We found that the duration of neurophysiological effects of MCS mirrors the duration of the behavioral effects (reduction in hyperalgesia) (**Task 1b,c**).

REPORTABLE OUTCOMES

1. Publications in peer reviewed Journals (attached in **Appendix**)
    - Davoody L, Quiton RL, Lucas JM, Keller A, Masri R. Conditioned Place Preference Reveals Tonic Pain in an Animal Model of Central Pain. J Pain. 2011; 12:868-74.
    - Lucas JM, Ji Y, Masri R. Motor Cortex Stimulation Reduces Hyperalgesia in an Animal Model of Central Pain. Pain. 2011; 152:1398-407.
2. Book Chapters
    - Masri R, Keller A. Chronic Pain Following Spinal Cord Injury. In: Frontiers in Spinal Cord and Spine Repair. Ed: Jandial R. Landes Bioscience. 2011; in press.
3. Abstracts
    - Lucas J, Qiuton R, Davoody L, Keller A, Masri R. Analgesic treatment relieves the tonic-aversive state in an animal model of central pain. 2010; Society for Neuroscience Meeting, Dan Diego, CA.
4. Meetings
    - Attended the 2011 International Conference on Spinal Cord Medicine and Rehabilitation as required by the CDMRP. Washington, District of Columbia.

CONCLUSION

In this report we provide evidence that electrical stimulation of the motor cortex (MCS) enhances spontaneous and evoked activity in the GABAergic nucleus zona incerta (ZI) in rodents with SCI-pain. The effects of MCS are mediated through direct action on ZI.

In addition, we provide evidence that MCS suppresses spontaneous and evoked activity in the posterior thalamus (PO). These findings are exciting; they are consistent with our overarching hypothesis that MCS alleviates pain by activating the incerto-thalamic circuit.

The findings of this study describe for the first time a novel pathway that is responsible for the amelioration of SCI-pain. In the coming year, we will continue to investigate this pathway to demonstrate that the suppression of activity in PO is due to MCS effects on ZI and that these neurophysiological changes can explain the reduction in hyperalgesia and pain after MCS.

REFERENCES

Bishop, GA, King, JS (1982) Intracellular horseradish peroxidase injections for tracing neural connections. In: Tracing Neural Connections with Horseradish Peroxidase (Mesulam, M-M, ed), pp 185–247. New York: John Wiley & Sons.

Davoody L, Quiton RL, Lucas JM, Ji Y, Keller A, Masri R. Conditioned place preference reveals tonic pain in an animal model of central pain. J Pain. 2011, 12:868-874.

Lucas JM, Ji Y, Masri R. Motor cortex stimulation reduces hyperalgesia in an animal model of central pain. Pain. 2011, 152:1398-1407.

Masri, R, Quiton, RL, Lucas, JM, Murray, PD, Thompson, SM, Keller, A (2009) Zona incerta: A role in central pain. J Neurophysiol, 102:181–191.

Swadlow, HA (1989) Efferent neurons and suspected interneurons in S-1 vibrissa cortex of the awake rabbit: receptive fields and axonal properties. J Neurophysiol, 62:288–308.

Swadlow, HA, Weyand, TG (1987) Corticogeniculate neurons, corticotectal neurons, and suspected interneurons in visual cortex of awake rabbits: receptive-field properties, axonal properties, and effects of EEG arousal. J Neurophysiol, 57:977–1001.

Trageser, JC, Burke, KA, Masri, RM, Li, Y, Sellers, L, Keller, A (2006) State-dependent gating of sensory inputs by zona incerta. J. Neurophysiol., 96:1456–1463.

Urbain, N, Deschenes, M (2007) Motor cortex gates vibrissal responses in a thalamocortical projection pathway. Neuron, 56:714–725.

Wang, G, Thompson, SM (2008) Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. J Neurosci, 28:11959–11969.

APPENDIX

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Conditioned Place Preference Reveals Tonic Pain in an Animal Model of Central Pain

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Abstract: **A limitation of animal models of central pain is their inability to recapitulate all clinical characteristics of the human condition. Specifically, many animal models rely on reflexive measures of hypersensitivity and ignore, or cannot assess, spontaneous pain, the hallmark characteristic of central pain in humans. Here, we adopt a conditioned place preference paradigm to test if animals with lesions in the anterolateral quadrant of the spinal cord develop signs consistent with spontaneous pain. This paradigm relies on the fact that pain relief is rewarding to animals, and has been used previously to show that animals with peripheral nerve injury develop tonic pain. With the use of 2 analgesic treatments commonly used to treat patients with central pain (clonidine infusion and motor cortex stimulation), we demonstrate that analgesic treatments are rewarding to animals with spinal cord lesions but not sham-operated controls. These findings are consistent with the conclusion that animals with spinal cord injury suffer from tonic pain.**

Perspective: ***The hallmark characteristic of central pain in humans is spontaneous pain. Animal models of central pain rely on reflexive measures of hypersensitivity and do not assess spontaneous pain. Demonstrating that animals with spinal cord injury suffer from tonic pain is important to study the etiology of central pain.***

Key words: *Spontaneous pain, motor cortex stimulation, posterior thalamus, rat, clonidine.*

A common consequence of spinal cord injury is the development of severe, debilitating chronic pain.[1,29,36] In patients, the pain manifests with a wide range of intensities and locations. It is usually persistent in the absence of an insult (spontaneous pain), and can present as hypersensitivity to painful stimuli (hyperalgesia) and hypersensitivity to normally innocuous stimuli (allodynia).[2] The etiology of the pain is unknown and is thought to be caused by maladaptive changes in the central nervous system.

Several animal models have been developed to study central pain, many of which focused on studying pain due to spinal cord injury. In all of these models, the location, the extent, and the means to produce injury vary. Some authors use controlled spinal contusions to mimic clinical traumatic injuries.[12,26,39] Others have used ischemic lesions,[9,10] or neurotoxic chemical injection into the spinal cord,[4,37] whereas some have used cuts to sever the spinal cord (hemisection),[5,6] or localized regions in the spinal cord (cordotomy).[31,32] Most of these models rely on measures of evoked pain and hypersensitivity, such as mechanical and thermal withdrawal thresholds. However, they commonly do not attempt to quantify spontaneous pain, which is the single most common and debilitating complaint from spinal cord injury patients.[8,29] Our aim was to assess whether animals with spinal cord injury suffer from spontaneous pain.

We have demonstrated recently that localized electrolytic lesions in the anterolateral quadrant of the spinal cord result in consistent, long-lasting mechanical and

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The authors of this paper have no financial or other conflicts of interest to declare.
Leyla Davoody and Raimi L. Quiton contributed equally to this work.
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thermal hyperalgesia.[19] Like other animal models of central pain, we relied on evoked measures of hypersensitivity to assess hyperalgesia and did not test if animals exhibit symptoms of spontaneous pain. Here, we employ a conditioned place preference paradigm described by King et al[14] to study tonic pain in animals. This approach takes advantage of the fact that pain relief is rewarding and, therefore, analgesic treatments should only be rewarding in the presence of pain.[14] We use the conditioned place preference paradigm combined with 2 treatments known to alleviate neuropathic pain (clonidine infusion or electrical stimulation of the motor cortex) to test if animals develop signs of spontaneous pain following spinal cord lesions. We demonstrate that lesioned animals, but not sham-operated controls, develop rapid preference to the analgesic treatment-paired chamber.

Methods

All procedures were approved by the University of Maryland Animal Care and Use Committee. Experiments were conducted according to institutional guidelines, federal regulations, and the guidelines of the International Association for the Study of Pain.

Protocol Overview

Twenty-eight adult female Sprague-Dawley rats (Harlan, IN) weighing 250 to 300 g, were used in this study, which was conducted over a 10-week period. Two experiments were conducted concurrently: 1) Drug group, to test the effect of analgesic drug administration (clonidine) on the conditioned place preference of animals with spinal cord injury (n = 11); and 2) Stimulation group, to test the effect of motor cortex stimulation on the conditioned place preference of animals with spinal cord injury (n = 17). In weeks 1 and 2, rats were habituated to handling and trained to stand with their forepaws on the experimenter's hand, allowing access to the hindpaws, as described in Ren.[25] During week 3, rats underwent behavioral tests to measure mechanical hindpaw withdrawal thresholds (see below). During week 4, rats underwent spinal lesion surgery to induce central pain or sham lesion surgery as a control and, for animals receiving motor cortex stimulation, to implant insulated platinum electrodes (see below). Weeks 5 and 6 involved further behavioral testing to measure mechanical hindpaw withdrawal thresholds and monitor the development of injury-related hyperalgesia. During week 7, rats in the clonidine/saline group underwent surgery to implant cannulae in the lateral ventricle for drug administration. Week 8 involved recovery from surgery and further testing of mechanical hindpaw withdrawal thresholds. The conditioned place preference protocol was conducted during weeks 9 and 10, along with further testing of mechanical hindpaw withdrawal thresholds.

Mechanical Hindpaw Withdrawal Threshold Testing

Mechanical hindpaw withdrawal thresholds were measured bilaterally using calibrated von Frey filaments (Stoelting, IL). Filaments with forces ranging from 10 to 180 g were applied to the dorsal surface of the hindpaw, based on studies demonstrating that threshold changes are more reliably and consistently detected at this site.[25] Each von Frey filament was applied 5 times to each hindpaw and the threshold was defined as the force at which the animal withdrew the paw to 3 or more of the stimuli (>50% response frequency). Animals were not restrained during testing. Rats underwent von Frey testing on 3 days in week 3 (before spinal or sham lesion surgery) to obtain baseline presurgical withdrawal thresholds, and every 7 days postlesion surgery for the duration of the study. Rats were also tested during the conditioned place preference protocol (week 10) to determine mechanical thresholds in the presence of intraventricular drug treatment or motor cortex stimulation (see below).

Surgical Procedures

Spinal Lesions

Fifteen adult female Sprague-Dawley rats underwent spinal lesion surgery, and 13 underwent sham lesion surgery during week 4 of the study. Eleven rats (n = 6 lesioned, n = 5 sham) underwent surgery to implant a cannula in the right lateral ventricle during week 7 of the study. Surgeries were conducted under strict aseptic conditions. Rats were anesthetized with ketamine/xylazine (100/8 mg/kg, ip) and placed on a thermo-regulated heating pad to maintain body temperature. For spinal lesions, a laminectomy was performed to expose the spinal cord between C6 and T2. A quartz-insulated platinum electrode (5-μm tip) was targeted unilaterally to the ventrolateral quadrant of the spinal cord, as described previously.[19,33] Current (10 μA for 10 seconds, repeated 4 times) was passed through the electrode to produce an electrolytic lesion (approximately .6 mm^3; lesion locations, .8 mm lateral from midline; depth, 2.1 mm). In some animals (n = 9), to produce larger spinal lesions, we modified our approach to produce 2 lesions, .4 mm apart (lesion locations, .8 mm and 1.2 mm lateral from midline; depth, 2.1 mm). However, the modification in the protocol had no effect on the consistency or features of the resultant hyperalgesia. Sham surgery was performed without laminectomy.

Implantation of Motor Cortex Stimulation Electrodes

In 17 animals ("stimulation group") and, concurrent with spinal lesion surgery, a longitudinal incision was made along the midline of the skull to expose bregma and lambda. The bone overlying the primary motor cortex (MI) was removed contralateral to the spinal lesion site. Custom-made epidural bipolar insulated platinum electrodes (diameter, 70 μm; exposed tip, 50 μm; distance between electrodes, 500 μm) were targeted to the MI contralateral to the site of spinal lesion using stereotaxic coordinates (A: 1.8 mm, L: 2 mm). These coordinates were obtained from pilot experiments using electrical microstimulation and from data obtained from our previously

published motor cortex mapping work.[34] This allowed us to reliably target the hindpaw representation of MI since the location of major subdivisions such as the forelimb or hindlimb areas in the rat motor cortex is somatotopic and consistent from animal to animal.[22] Motor cortex stimulation electrodes were attached to amphenol pins to facilitate connection to the isolated pulse stimulator (A-M Systems; Sequim, WA). Electrodes were fixed in place using 4 bone screws and acrylic resin. At the end of surgery, the wound edges were approximated and sutured to achieve primary closure.

Cannula Implantations

In 11 animals ("drug group"), a craniotomy was performed to expose the brain over the right lateral ventricle in week 7. A guide cannula was advanced to the ventricle and fixed in place using dental resin.

Postoperative Care

The analgesic buprenorphine (.05 mg/kg) was administered every 12 hours for 24 hours postoperatively following spinal lesion surgeries and motor cortex electrode implantation, and every 12 hours for 3 days postoperatively following cannula implantations.

Conditioned Place Preference Protocol

Conditioned place preference testing was conducted using a custom-built, automated 2-chamber box. The walls of 1 chamber were white with horizontal black stripes and the walls of the other chamber were white with vertical black stripes. We used chambers with striped walls to ensure that rats would not strongly prefer 1 chamber over the other, as they would if we had used a more traditional conditioned place preference box with 1 dark-walled chamber and 1 light-walled chamber.

Rats were habituated to the conditioned place preference box for 3 days during week 9 of the study. On each habituation day, rats were permitted to move freely between the 2 chambers for 30 minutes. On day 4 of week 9, a preconditioning preference test was conducted in which rats were permitted to move freely between the 2 chambers for 15 minutes and time spent in each chamber was recorded to determine each rat's preference.

After habituation and the preconditioning preference test (week 9), rats underwent a 3-day conditioning phase in week 10 of the study. Two sessions were conducted on each day, at least 6 hours apart.

In 1 session, the animals were placed in the chamber that they demonstrated preference for during the preconditioning test. They spent 30 minutes in that chamber where rats in the drug group received an intraventricular microinjection of vehicle (5-μl saline followed by 10-μl saline flush); rats in the stimulation group received sham motor cortex stimulation (wires attached but no current passed).

In the other daily session, the animals were placed for 30 minutes in the chamber that they did not prefer during the preconditioning test. Here, rats in the drug group received an intraventricular microinjection of clonidine, an alpha 2-adrenergic agonist (5 μl [2 mg/mL] followed by 10-μl saline flush), and those in the stimulation group received motor cortex stimulation (50 μA, 50 Hz, for 30 minutes). We used intraventricular clonidine in these experiments because it has been shown previously to reduce tonic and evoked pain in animals with peripheral neuropathic pain without affecting normal uninjured animals.[14]

Drug or motor cortex stimulation treatment order was randomized for each rat. That is, some days the rat received vehicle in the first session while other days the rat received drug in the first session. Mechanical hindpaw withdrawal thresholds were measured 1 hour following saline/clonidine intraventricular injection or immediately after the end of motor cortex stimulation.

One day after the conditioning phase, a postconditioning place preference test was conducted in which rats received no drug treatment and were permitted to move freely between the 2 chambers for 15 minutes. Time spent in each chamber was recorded to determine each rat's chamber preference.

Data Analysis

Statistical analyses were performed with SigmaStat (Aspire Software International; Ashburn, VA). To test whether mechanical hindpaw withdrawal thresholds changed over time after surgery, data from spinal-lesioned rats and sham-lesioned rats were analyzed separately with the Friedman test. To test the effects of clonidine or motor cortex stimulation treatment on mechanical hyperalgesia, data from spinal-lesioned rats and sham-lesioned rats were analyzed separately with the Wilcoxon Signed Ranks test. Conditioned place preference test results were analyzed using a 2-way analysis of variance (group, conditioning) with repeated measures on 1 factor (group), followed by a post hoc Fisher least significant difference (LSD) test to compare individual factors. The significance level was set at $P < .05$ for all tests.

Results

Animals in the Drug Group Develop Mechanical Hyperalgesia Following Spinal Lesions

We and others have previously shown that rats with spinal cord lesions develop behavioral signs consistent with central pain, including mechanical and thermal hyperalgesia caudal to the lesion site.[19,27,32] Consistent with the literature, all spinal-lesioned rats in the clonidine/saline treatment group showed a significant decrease in mechanical hindpaw withdrawal thresholds bilaterally within 7 days of the lesion surgery (Fig 1A). Mechanical thresholds decreased from 93.3 g (SD 16; median 100; range 60–100; n = 6) to 55.3 g (SD 28; median 60; range 26–100; $P < .001$, Friedman). Sham surgery (n = 5) had no effect on mechanical withdrawal thresholds on either the ipsilateral or contralateral hindpaw (Fig 1A). Each animal tested had identical withdrawal thresholds on the ipsilateral and contralateral hindpaw at every time point. As a result, Fig 1A shows the

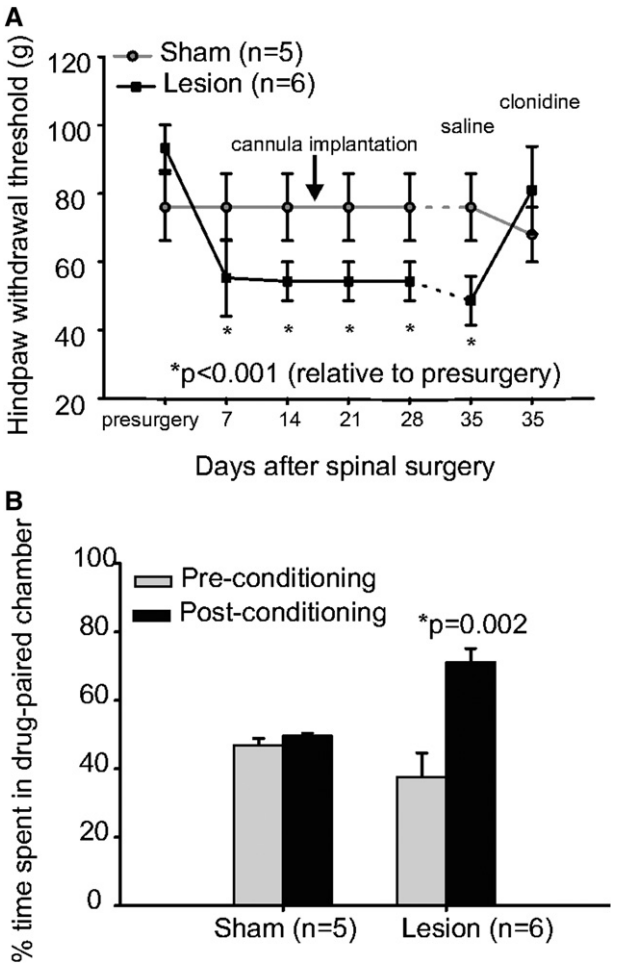

Figure 1. Intraventricular administration of clonidine reverses mechanical hyperalgesia and unmasks a tonic aversive state. A. Animals in the clonidine/saline group develop mechanical hyperalgesia following spinal cord lesions (n = 6; $P < .001$) while sham surgery had no effect on mechanical withdrawal thresholds (n = 5). Intraventricular administration of clonidine (5 μl [2mg/mL] followed by 10-μl saline flush) reversed mechanical hyperalgesia in animals with spinal cord lesions. The administration of an equivalent volume of saline had no effect of mechanical withdrawal thresholds. B. The percentage of time spent in the drug paired chamber is shown. Animals with spinal cord injury (n = 6) prefer the chamber where they receive clonidine treatment ($P = .002$). Clonidine had no effect on chamber preference of sham-operated controls (n = 5). Error bar = SEM. *Statistically significant difference.

behavioral data for the contralateral hindpaw; results for the ipsilateral hindpaw were the same and are therefore not shown.

Clonidine Reverses Mechanical Hyperalgesia in Animals With Spinal Lesions

Intraventricular injection of clonidine (2 mg/mL) at 35 days after spinal lesion surgery reversed hyperalgesia in lesioned animals (Fig 1A), returning mechanical withdrawal thresholds to prespinal lesion values (presurgery: mean 93.3 g, SD 16, median 100, range 60–100; clonidine at 35 days: mean 81 g, SD 31, median 100, range 26–100; $P = 1.0$, Wilcoxon). The infusion of an equivalent volume of saline at 35 days had no effect on mechanical hyperalgesia in these animals (mean 48.7 g, SD 18, median 60, range 26–60; $P = .03$, compared with presurgical values, Wilcoxon; Fig 1A). Clonidine and saline treatments had no effect on mechanical hindpaw withdrawal thresholds in sham-lesioned animals ($P > .05$). These findings are consistent with previous studies demonstrating that clonidine is effective in reducing mechanical hyperalgesia in animal models of neuropathic pain and that it can be used to examine behaviors related to chronic neuropathic pain.[7,14]

Clonidine Unmasks the Tonic Aversive State in Animals With Spinal Lesions

Before conditioning, both sham-operated controls and spinal-lesioned animals showed a slight but not statistically significant preference for the vertically striped chamber (Fig 1B). Sham animals spent an average of 46.9% (SD 4, n = 5) of the 15-minute test period in the horizontally striped chamber, while spinal-lesioned animals spent an average of 37.6% (SD 17, n = 6) of the test period in the horizontally striped chamber. During the conditioning phase, all animals received intraventricular infusions of clonidine (2 mg/mL) and were then restricted to the horizontally striped chamber for 30 minutes. Saline injections were paired with restriction in the vertically striped chamber (repeated on 3 days). After this conditioning paradigm, animals with spinal cord lesions, but not sham animals (animals without spinal cord injury), developed a strong and significant preference for the clonidine paired chamber (Fig 1B), spending an average of 71.1% (SD 10) of the 15-minute test period in the drug-paired horizontally striped chamber ($P = .002$, post hoc Fisher LSD; $F = 6.91$, $P = .03$ for group $\times$ conditioning interaction, 2-way ANOVA). The preference of the sham animals remained unchanged, with this group spending an average of 49.6% (SD 2) of the test period in the horizontally striped chamber ($P > .05$, post hoc Fisher LSD). The findings that spinal-lesioned animals, but not shams, prefer the chamber in which analgesia is provided, suggests that clonidine unmasks a tonic aversive state.

Motor Cortex Stimulation Unmasks the Tonic Aversive State in Animals With Spinal Lesions

Like the first group of animals in this study, these spinal-lesioned rats developed a significant decrease in mechanical hindpaw withdrawal thresholds bilaterally (Fig 2A). Mechanical thresholds decreased from 121.2 g (SD 39, median 100, range 60–180, n = 9) to 60.0 g at week 8 (SD 17.5, median 60, range 26–100; $P < .001$, Friedman). Sham surgery (n = 8) had no effect on mechanical withdrawal thresholds on either the ipsilateral (Fig 2A) or contralateral hindpaw (data not shown).

Consistent with our previous findings (Lucas, 2010), motor cortex stimulation (50 μA, 50 Hz, 300-μs square pulse, 30-minute duration) 63 days after spinal lesion surgery significantly reduced the hyperalgesia in animals with spinal cord injury (Stimulation on: mean 80 g, SD 21, median 80, range 60–100; Stimulation off: mean

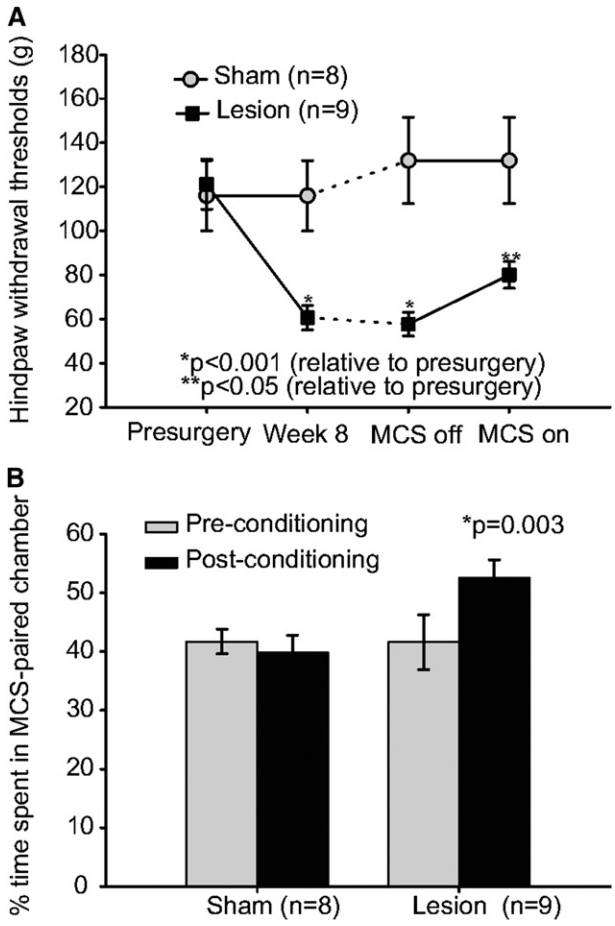

Figure 2. Motor cortex stimulation reduces mechanical hyperalgesia and unmasks a tonic aversive state. A. Like the clonidne/saline group, animals in the motor cortex stimulation group developed mechanical hyperalgesia following spinal cord lesions (n = 9; P = .001). Mechanical withdrawal thresholds in animals receiving sham surgery were not significantly different from presurgical values. Motor cortex stimulation (50 μA, 50 Hz, for 30 minutes) reduced mechanical hyperalgesia in animals with spinal cord lesions (P = .04), but had no effect on sham-operated controls. B. Animals with spinal cord lesions (n = 9) preferred the chamber where they received motor cortex stimulation (P = .003). Motor cortex stimulation had no effect on the preference of sham operated controls. Error bar = SEM. *Statistically significant difference. MCS, motor cortex stimulation.

57.7 g, SD 19, median 60, range 26–100; P = .04, Wilcoxon, n = 9). Unlike the clonidine treatment, the reduction in mechanical hyperalgesia after motor cortex stimulation was not complete and mechanical threshold values did not return to presurgery levels (P = .04, Wilcoxon). Motor cortex stimulation in sham-lesioned animals (n = 8) had no effect on mechanical hindpaw withdrawal thresholds (P > .05, Fig 2A).

To test if motor cortex stimulation unmasks the tonic aversive state in animals with anterolateral spinal cord lesions, we used the conditioned place preference test. Before conditioning, both sham and spinal-lesioned animals showed a slight but not statistically significant preference for the vertically striped chamber (Fig 2B). Sham animals spent an average of 41.7% (SD 5, n = 8) of the 15-minute test period in the horizontally striped chamber, while spinal-lesioned animals spent an average of 39.9% (SD 10, n = 9) of the test period in the horizontally striped chamber. During the conditioning phase, all animals received motor cortex stimulation (50 μA, 50 Hz, 300-μs square pulse, 30-minute duration) while restricted in the horizontally striped chamber, and received sham stimulation while restricted in the vertically-striped chamber (repeated on 3 days). After this conditioning paradigm, lesioned animals, but not sham animals, developed a preference to the motor cortex stimulation-paired chamber (Fig 2B), spending an average of 52% (SD 9) of the 15-minute test period in the motor cortex stimulation-paired chamber (P = .003, post hoc Fisher LSD; F = 4.34, P = .04 for group $\times$ conditioning interaction, 2-way ANOVA). The preference of the sham animals remained unchanged, with this group spending an average of 42% (SD 12) of the test period in the horizontally striped chamber (P > .05, post hoc Fisher LSD). Findings from the conditioned place preference test suggest that motor cortex stimulation reduces tonic pain.

Discussion

This study was designed to test whether animals with anterolateral spinal cord lesions suffer from spontaneous pain. We found that animals with spinal cord injury develop mechanical hyperalgesia that can be attenuated by treatments commonly used for patients with central neuropathic pain: clonidine or motor cortex stimulation. Using the conditioned place preference test, we further demonstrate that these treatments unmask a tonic aversive state suggesting that these animals exhibit signs of spontaneous pain.

Signs of Spontaneous Pain in Animals With Spinal Cord Lesions

The conditioned place preference test, or modifications of it, is commonly used to study the motivational effects of drugs and pain on animals.[13,15] King et al[14] adopted the conditioned place preference paradigm to investigate if animals with peripheral neuropathic pain suffer from spontaneous pain. They demonstrated that animals with spinal nerve ligation, but not sham-operated controls, rapidly develop a preference to the clonidine-paired chamber. King et al[14] posited that clonidine administration results in the removal of a tonic state, suggesting that the animals experience pain relief. These findings led King et al to conclude that animals with SNL suffer from tonic pain.[14]

In our animal model of central pain, and similar to King et al, intraventricular clonidine administration resulted in negative reinforcement whereby lesioned animals preferred the drug-paired chamber while clonidine was not rewarding in the absence of injury. Therefore, these findings suggest that animals with spinal cord injury suffer from tonic pain. Clonidine not only unmasked the presence of a tonic pain component, but also reversed mechanical hyperalgesia in animals with spinal cord lesions. These findings are consistent with previous reports that clonidine reverses mechanical and thermal hyperalgesia in animal models of neuropathic pain.[7,14] Another treatment for neuropathic pain—motor cortex stimulation—also resulted in negative reinforcement in

animals with spinal cord lesions but not in controls. These findings further support the notion that spinal cord-lesioned animals suffer from tonic pain.

Spontaneous pain is difficult to assay in animals and especially rodents because they do not display behaviors or postures that reflect the presence of mild-to-moderate pain.[21,28] Current animal models of central pain are unable to demonstrate that animals with spinal cord injury suffer from spontaneous pain. Previous investigations in animals relied heavily on observations of overgrooming/autotomy, licking, guarding, and vocalization.[30] These behaviors have been criticized as unreliable and nonspecific.[20,35] The conditioned place preference paradigm offers a suitable supplement to these behaviors especially since it specifically measures cognitive, motivated preference, and pain is an emotional cognitive experience.

Neuropathological Basis of Ongoing Pain Following Spinal Cord Injury

Spinal cord injury can result in maladaptive plastic changes throughout the neural axis. In the spinal cord following injury, there is massive reorganization and sprouting in primary afferents.[5,6] Injury causes elevated concentrations of excitatory amino acids[18] in the extracellular space and dorsal horn neurons show increased spontaneous activity.[10,11] In the thalamus, spinal cord injury results in increased activity, increased spike bursts, and changes in glial activation.[33,40] We have demonstrated that spontaneous activity of PO and SI neurons are dramatically increased (PO, ~30 fold increase; S1, ~3 fold) in animals with spinal lesions when compared to sham-operated controls.[19,24] The change in spontaneous activity of thalamic and cortical neurons may contribute to the tonic pain observed in our animal model of spinal cord injury pain.

In humans with spinal cord injury and chronic pain, functional imaging studies reveal significant changes in blood flow in the thalamus during rest[3,23] and electrophysiological recordings reveal abnormal spontaneous discharges in thalamic neurons.[16,17] These maladaptive changes may contribute to the pathogenesis of spontaneous pain in humans. However, the underlying mechanisms remain to be elucidated.

Here, we present findings that suggest that animals with spinal cord injury exhibit signs of spontaneous pain. The presence of tonic pain and hyperalgesia, the small size of spinal damage when compared to other animal models of spinal cord injury,[5,10,27,32,38] the reduced morbidity and the high percentage of animals developing hyperalgesia after lesions (94%)[19] make this model ideal to study the neurobiological substrates responsible for the development of central pain.

References

1. Beric A: Post-spinal cord injury pain states. Pain 72: 295-298, 1997

2. Boivie J: Central pain, in McMahon S, Koltzenburg M (eds): Wall and Melzack's Textbook of Pain, 5th ed. Oxford, UK, Churchill Livingstone, 2005, pp 1057-1074

3. Canavero S, Bonicalzi V: The neurochemistry of central pain: Evidence from clinical studies, hypothesis and therapeutic implications. Pain 74:109-114, 1998

4. Caudle RM, Perez FM, King C, Yu CG, Yezierski RP: N-methyl-D-aspartate receptor subunit expression and phosphorylation following excitotoxic spinal cord injury in rats. Neurosci Lett 349:37-40, 2003

5. Christensen MD, Everhart AW, Pickelman JT, Hulsebosch CE: Mechanical and thermal allodynia in chronic central pain following spinal cord injury. Pain 68:97-107, 1996

6. Christensen MD, Hulsebosch CE: Chronic central pain after spinal cord injury. J Neurotrauma 14:517-537, 1997

7. Feng X, Zhang F, Dong R, Li W, Liu J, Zhao X, Xue Q, Yu B, Xu J: Intrathecal administration of clonidine attenuates spinal neuroimmune activation in a rat model of neuropathic pain with existing hyperalgesia. Eur J Pharmacol 614:38-43, 2009

8. Finnerup NB, Johannesen IL, Sindrup SH, Bach FW, Jensen TS: Pain and dysesthesia in patients with spinal cord injury: A postal survey. Spinal Cord 39:256-262, 2001

9. Hao JX, Xu XJ: Treatment of a chronic allodynia-like response in spinally injured rats: Effects of systemically administered excitatory amino acid receptor antagonists. Pain 66:279-285, 1996

10. Hao JX, Xu XJ, Aldskogius H, Seiger A, Wiesenfeld-Hallin Z: Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. Pain 45:175-185, 1991

11. Hao JX, Xu XJ, Yu YX, Seiger A, Wiesenfeld-Hallin Z: Transient spinal cord ischemia induces temporary hypersensitivity of dorsal horn wide dynamic range neurons to myelinated, but not unmyelinated, fiber input. J Neurophysiol 68:384-391, 1992

12. Hulsebosch CE, Hains BC, Crown ED, Carlton SM: Mechanisms of chronic central neuropathic pain after spinal cord injury. Brain Res Rev 60:202-213, 2009

13. Johansen JP, Fields HL, Manning BH: The affective component of pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. Proc Natl Acad Sci U S A 98: 8077-8082, 2001

14. King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F: Unmasking the tonic-aversive state in neuropathic pain. Nat Neurosci 12: 1364-1366, 2009

15. LaBuda CJ, Fuchs PN: A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. Exp Neurol 163:490-494, 2000

16. Lenz FA, Kwan HC, Dostrovsky JO, Tasker RR: Characteristics of the bursting pattern of action potentials that occurs in the thalamus of patients with central pain. Brain Res 496: 357-360, 1989

17. Lenz FA, Tasker RR, Dostrovsky JO, Kwan HC, Gorecki J, Hirayama T, Murphy JT: Abnormal single-unit activity

recorded in the somatosensory thalamus of a quadriplegic patient with central pain. Pain 31:225-236, 1987

18. Liu D, Thangnipon W, McAdoo DJ: Excitatory amino acids rise to toxic levels upon impact injury to the rat spinal cord. Brain Res 547:344-348, 1991

19. Masri R, Quiton RL, Lucas JM, Murray PD, Thompson SM, Keller A: Zona incerta: A role in central pain. J Neurophysiol 102:181-191, 2009

20. Mogil JS: Animal models of pain: Progress and challenges. Nat Rev Neurosci 10:283-294, 2009

21. Morton DB, Griffiths PH: Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet Rec 116:431-436, 1985

22. Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, Terreberry RR: The organization of the rat motor cortex: A microstimulation mapping study. Brain Res 396: 77-96, 1986

23. Peyron R, Garcia-Larrea L, Gregoire MC, Convers P, Richard A, Lavenne F, Barral FG, Mauguiere F, Michel D, Laurent B: Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case. Pain 84:77-87, 2000

24. Quiton RL, Masri R, Thompson SM, Keller A: Abnormal activity of primary somatosensory cortex in central pain syndrome. J Neurophysiol 104:1717-1725, 2010

25. Ren K: An improved method for assessing mechanical allodynia in the rat. Physiol Behav 67:711-716, 1999

26. Scheff SW, Rabchevsky AG, Fugaccia I, Main JA, Lumpp JEJ: Experimental modeling of spinal cord injury: Characterization of a force-defined injury device. J Neurotrauma 20:179-193, 2003

27. Siddall P, Xu CL, Cousins M: Allodynia following traumatic spinal cord injury in the rat. Neuroreport 6: 1241-1244, 1995

28. Stasiak KL, Maul D, French E, Hellyer PW, VandeWoude S: Species-specific assessment of pain in laboratory animals. Contemp Top Lab Anim Sci 42:13-20, 2003

29. Stormer S, Gerner HJ, Gruninger W, Metzmacher K, Follinger S, Wienke C, Aldinger W, Walker N, Zimmermann M, Paeslack V: Chronic pain/dysaesthesiae in spinal cord injury patients: Results of a multicentre study. Spinal Cord 35:446-455, 1997

30. Vierck CJ, Siddall P, Yezierski RP: Pain following spinal cord injury: Animal models and mechanistic studies. Pain 89:1-5, 2000

31. Vierck CJJ, Greenspan JD, Ritz LA: Long-term changes in purposive and reflexive responses to nociceptive stimulation following anterolateral chordotomy. J Neurosci 10: 2077-2095, 1990

32. Vierck C Jr, Light A: Effects of combined hemotoxic and anterolateral spinal lesions on nociceptive sensitivity. Pain 83:447-457, 1999

33. Wang G, Thompson SM: Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: Thalamic hyperexcitability after spinothalamic tract lesions. J Neurosci 28:11959-11969, 2008

34. Weiss DS, Keller A: Specific patterns of intrinsic connections between representation zones in the rat motor cortex. Cerebral Cortex 4:205-214, 1994

35. Woolf CJ: Long term alterations in the excitability of the flexion reflex produced by peripheral tissue injury in the chronic decerebrate rat. Pain 18:325-343, 1984

36. Yezierski RP: Pain following spinal cord injury: Pathophysiology and central mechanisms. Prog Brain Res 129: 429-449, 2000

37. Yezierski RP, Liu S, Ruenes GL, Kajander KJ, Brewer KL: Excitotoxic spinal cord injury: Behavioral and morphological characteristics of a central pain model. Pain 75: 141-155, 1998

38. Yezierski RP, Park SH: The mechanosensitivity of spinal sensory neurons following intraspinal injections of quisqualic acid in the rat. Neurosci Lett 157:115-119, 1993

39. Yoon YW, Dong H, Arends JJ, Jacquin MF: Mechanical and cold allodynia in a rat spinal cord contusion model. Somatosens Mot Res 21:25-31, 2004

40. Zhao P, Waxman SG, Hains BC: Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. J Neurosci 27:8893-8902, 2007

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Motor cortex stimulation reduces hyperalgesia in an animal model of central pain

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ABSTRACT

Electrical stimulation of the primary motor cortex has been used since 1991 to treat chronic neuropathic pain. Since its inception, motor cortex stimulation (MCS) treatment has had varied clinical outcomes. Until this point, there has not been a systematic study of the stimulation parameters that most effectively treat chronic pain, or of the mechanisms by which MCS relieves pain. Here, using a rodent model of central pain, we perform a systematic study of stimulation parameters used for MCS and investigate the mechanisms by which MCS reduces hyperalgesia. Specifically, we study the role of the inhibitory nucleus zona incerta (ZI) in mediating the analgesic effects of MCS. In animals with mechanical and thermal hyperalgesia, we find that stimulation at 50 μA, 50 Hz, and 300 μs square pulses for 30 minutes is sufficient to reverse mechanical and thermal hyperalgesia. We also find that stimulation of the ZI mimics the effects of MCS and that reversible inactivation of ZI blocks the effects of MCS. These findings suggest that the reduction of hyperalgesia may be due to MCS effects on ZI.

1. Introduction

Central pain is defined as pain resulting from primary lesion or dysfunction in the central nervous system [34]. The pain is unrelenting, and refractory to pharmacologic treatments [1]. Electrical stimulation of the primary motor cortex was introduced in 1991 for the treatment of central pain syndrome (CPS) [61]. Since then its use has been extended for the treatment of several neuropathic pain conditions. These include trigeminal deafferentation pain [18], postherpetic neuralgia [5], brachial plexus, and phantom limb pain [53].

Stimulation of other brain structures has also been used for the treatment of neuropathic pain. These include the internal capsule [37], the periaqueductal gray-periventricular gray complex [25], and the thalamus [38]. However, motor cortex stimulation (MCS) is more effective and more advantageous because of the low occurrence of complications [9], the lower propensity to cause seizures [2,15], and the ability to apply it noninvasively using repetitive transcranial magnetic stimulation [27].

Human studies report mixed outcomes after MCS and success rates vary. MCS relieves pain in approximately 50% of patients [21,31], while studies involving only patients with CPS report success rates as high as 77% [60,61] (but see [14]). The mixed success rates and the mixed outcomes after MCS are a reflection of the complexity and variability of neuropathic pain conditions. Adding to the variability of MCS efficacy is the lack of standardized surgical, stimulation, and treatment protocols [20]. In human studies, stimulation parameters vary (intensities: 1 to 8 V, frequencies: 15 to 130 Hz, pulse duration: 60–500 μs), as do stimulation protocols (MCS on range: 15 minutes–3 hours, MCS off: 2–24 hours; reviewed in [31]). Because of this variability, it is not clear which stimulus parameters are critical for MCS to be successful.

Pain relief occurs almost immediately after onset of MCS and persists after the stimulation has stopped. Like stimulation parameters, the duration of effect after cessation of stimulation ("post effect") is rarely systematically examined, and reported values vary among studies. In some reports, these post effects were minimal and lasted for only 5–10 minutes [54,55,61]. In others, post effects varied from 45 minutes–2 hours [45], 3–5 hours [56], and even up to 1–3 days [40,41]. The variability in post effect duration is also a reflection of the various parameters used, various conditions predisposing for neuropathic pain, and different stimulation techniques.

Here, we take advantage of an animal model of central pain to systematically test a large parameter space of stimulus conditions. We determine the stimulus parameters that are effective in reducing hyperalgesia and study the mechanisms of increased inhibition in the thalamus following MCS.

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We have recently shown in an animal model of central pain that there is abnormally high neuronal activity in the posterior thalamic nucleus and that this increased activity and hyperalgesia is correlated with reduced activity in the inhibitory nucleus zona incerta (ZI) [33]. Because the motor cortex sends dense projections to the ventral division of ZI [35,62], we hypothesized that MCS reduces hyperalgesia by increasing activity in the ZI.

2. Methods

2.1. General surgical procedures

All procedures were conducted according to Animal Welfare Act regulations and Public Health Service guidelines. Strict aseptic surgical procedures were used, according to the guidelines of the International Association for the Study of Pain, and approved by the Baltimore College of Dental Surgery Animal Care and Use Committee. Twenty-six adult female Sprague-Dawley rats weighing 250–300 g were used in this study. Animals were anesthetized with ketamine/xylazine (100/8 mg/kg, intraperitoneal) and placed into a stereotaxic frame. Animals were then placed on a thermoregulated heating pad and respiratory rate, corneal reflex, and tail pinch response were monitored and used to ensure that animals were sufficiently anesthetized. Additional anesthesia (10 mg/kg intraperitoneal, diluted ketamine 1:10 in saline) was administered when needed. Local anesthetic (2% lidocaine) was applied to surgery sites before procedures began.

After the end of the surgical procedure, animals were left to recover on a thermoregulated heated pad and the analgesic buprenorphine (0.05 mg/kg) was administered every 12 hours for 24 hours postoperatively (2 doses total).

2.2. Spinal lesions

A midline, inch-long longitudinal incision overlying the area of C2-T2 was made using a #11 scalpel. The muscles were dissected under a dissecting microscope with blunt scissors to expose vertebrae C6 and C7. A laminectomy to expose the spinal cord immediately rostral to C7 was preformed using rongeurs, and the dura covering the exposed spinal cord was removed. A quartz-insulated platinum electrode (5-μm tip) was targeted to the anterolateral quadrant on one side of the spinal cord. In our previous publications, we used only one electrolytic lesion to injure the spinal cord [33,49]. To produce larger spinal lesions, we modified our approach and used DC current (10 μA for 10 seconds, repeated 4 times) to produce 2 lesions 0.4 mm apart (lesion locations: 0.8 mm and 1.2 mm lateral from midline; depth: 2.1 mm). However, the modification in the protocol had no effect on the consistency or features of the resultant hyperalgesia. After the completion of surgery, the incision sites were approximated and sutured in layers.

2.3. Motor cortex electrode implantation (n = 18)

Concurrent with spinal lesion surgery, a midline longitudinal incision was made along the midline of the skull to expose bregma and lambda. The bone overlying the primary motor cortex (M1) was removed contralateral to the spinal lesion site. Custom-made epidural bipolar insulated platinum electrodes (diameter: 70 μm, exposed tip: 50 μm, distance between electrodes: 500 μm) were targeted to the M1 contralateral to the site of spinal lesion using stereotaxic coordinates (A: 1.8 mm, L: 2 mm [43]). These coordinates were obtained from pilot experiments using electrical microstimulation and from data obtained from previously published motor cortex mapping work done by our collaborator, Dr. Asaf Keller [70]. This allowed us to reliably target the hind paw representation of M1, especially since the location of major subdivisions such as the forelimb or hindlimb areas in the rat motor cortex are somatotopic and consistent from animal to animal [39]. MCS electrodes were attached to amphenol pins to facilitate connection to the isolated pulse stimulator (A-M Systems, Sequim, WA, USA). Electrodes were fixed in place using 4 bone screws and acrylic resin.

2.4. Zona incerta electrode implantation (n = 8)

Concurrent with spinal lesion surgery, 8 rats received custom-made bipolar insulated stainless steel electrodes (diameter: 139 μm, exposed tip: 75 μm, distance between electrodes: 280 μm) implanted in ZI contralateral to the spinal lesion, targeted using stereotaxic coordinates (A: −3.6 mm, L: 2.8 mm, D: 7.3 mm, [43]). Briefly, a longitudinal incision was made along the midline of the skull to expose bregma and lambda. Bone overlying ZI was removed and the electrodes lowered 7.3 mm over the course of 20 minutes. Electrodes were fixed in place using 4 bone screws and acrylic resin.

2.5. Behavioral confirmation of hyperalgesia

To minimize anxiety, the animals were habituated for 1 week before behavioral testing and surgery. The animals were trained to stand upright with forepaws on the experimenter's hand, as described by Ren [50]. Testing of mechanical thresholds and thermal withdrawal latencies was performed on 3 consecutive days before the spinal lesion surgery, and on postsurgical days 3, 7, 14, and 21 to confirm the development of hyperalgesia.

To assess mechanical thresholds, calibrated von Frey filaments (Stoelting Co, Wood Dale, IL, USA) were applied in ascending order to the hind paws. We applied the filaments to the *dorsal* surface of the paws based on studies demonstrating that the dorsal approach more reliably and consistently detects threshold changes [50]. Mechanical withdrawal threshold was defined as the force at which the animal withdrew the paw to 3 of 5 stimuli delivered.

To assess thermal withdrawal latency, an analgesia meter with a moveable infrared heat source (IITC, Life Science Inc, Woodland Hills, CA) was used to apply radiant heat to the ventral surface of the hind paws as described in Hargreaves et al. [26]. Rats were acclimated to the test chambers (plexiglass boxes 17 [d] × 69 [l] × 14 [h] cm) for 30 minutes before testing. Latency to withdraw was recorded and thermal thresholds were computed as the average latency to withdraw across 3 trials.

2.6. Motor cortex stimulation

Before MCS was initiated, animals (n = 15) were habituated in stimulation chambers and handled to minimize anxiety. Baseline mechanical thresholds and thermal withdrawal latencies were obtained (see above) before MCS was performed. The stimulating electrodes were connected to a stimulator (A-M Systems) and stimulation parameters were varied. Stimulation intensity ranged from 0 to 75 μA, frequency from 0 to 75 Hz, and duration from 0 to 90 minutes. A 300-μs square pulse was used for all experiments. MCS effects were tested with von Frey filaments and a radiant heat source immediately after the end of MCS stimulation and every 30 minutes thereafter until mechanical thresholds returned to baseline values. Each animal was stimulated no more than once daily, and stimulation was repeated at least 3 times. For sham stimulation, animals were connected to the stimulator and placed in the test chambers but no current was passed.

During electrical stimulation, animals remained in the test chamber and were under constant observation. Stimulation did

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not produce any visible muscle twitches and the animals showed no signs of distress. We obtained all behavioral responses after the end of MCS stimulation because previous studies in humans suggest that the neural mechanisms responsible for long-term analgesia occur (and are most readily observable) after MCS, rather than during the stimulation period [44].

2.7. Zona incerta stimulation

Eight animals with mechanical and thermal hyperalgesia received electrical stimulation in ZI. Similar to MCS, the animals were handled and habituated in test chambers before stimulation. Postspinal lesion thresholds were obtained using the behavioral metrics described above. Over the course of 2 weeks, each animal was tested immediately after the termination of incertal stimulation on each of at least 3 days (intensity: 25 μA, frequency: 50 Hz, duration: 15 minutes, 300-μs square pulse) and 3 days of sham stimulation in a randomized fashion. After completion of ZI stimulation experiments, electrolytic lesions were made at the site of the electrode to confirm placement.

2.8. Zona incerta inactivation

In a subset of animals (n = 6) and concurrent with M1 electrode implantation, a microdialysis probe (CMA Microdialysis, Solna, Sweden) was implanted into the ventral portion of ZI (stereotaxic coordinates: A: −3.6 mm, L: 2.8 mm, D: 7.1 mm [43]). After recovery, behavioral testing to confirm the development of hyperalgesia, and behavioral testing to obtain reliable baseline values for efficacy of MCS, the microdialysis probe was used to administer lidocaine (2%), muscimol (200 μM), or saline to ZI. A total of 50 μL was administered over 20 minutes (beginning 5 minutes before MCS and continuing through the first 15 minutes of MCS) at a rate of 2.5 μL/min. Over the course of 2 weeks, infusion of drugs or saline was repeated 3 times per animal, and the data reported represent the average of these trials. In all animals, testing for changes in mechanical thresholds was performed after the termination of MCS. At the end of the experiments, the animals were perfused to identify the location of the microdialysis probes.

2.9. Histology

The animals were deeply anesthetized with sodium pentobarbital (60 mg/kg). The animals were perfused transcardially with buffered saline followed by 4% buffered paraformaldehyde. We obtained coronal brain sections (80 μm thick) and Nissl-stained them. The sections were examined under the microscope to identify stimulation sites, lesion sites, and probe implant location.

2.10. Data analysis

2.10.1. Confirmation of hyperalgesia

To determine that mechanical and thermal thresholds dropped significantly after spinal lesion, a Wilcoxon signed-rank test was performed using the average of 3 presurgical baseline trials and the average of at least 2 postsurgical trials. All lesioned animals exhibited significant reductions in withdrawal thresholds and thermal withdrawal latencies following spinal cord lesions.

2.10.2. Examining various stimulation protocols and parameters

The effects of differing stimulation trains (theta burst, intermittent theta burst, and continuous-pulse stimulation) were examined using a Friedman test followed by a modified Dunnett's post hoc.

The effect of varying MCS parameters (intensity, frequency, and duration) was tested using the Kruskal–Wallis test followed by a Dunn's post hoc test. To test for correlation between stimulus duration and the duration of post effects, Spearman's rho (ρ) test was performed.

2.10.3. ZI stimulation and inactivation

Pre- and post-ZI stimulation thresholds were tested using a Wilcoxon signed-rank test, while the effect of ZI inactivation was tested with a Kruskal–Wallis test.

In all experiments performed, we determined the appropriate sample size by performing a power analysis using $\alpha = 0.05$ and power = 0.85. All data were analyzed using SigmaStat (Aspire Software International, Ashburn, VA, USA) and presented as means ± SD. In all experiments, $P < 0.05$ was considered significant.

3. Results

3.1. Animals with spinal lesions develop hyperalgesia

In this project, we adopted a rodent model of central pain induced by spinal cord lesions [33,69]. In these animals, 14 days after spinal lesions, mechanical thresholds significantly reduced from 137.40 ± 45.73 g (mean ± SD; hind paw ipsilateral to lesion) and 127.30 ± 37.11 g (contralateral) before surgery to 58.29 ± 15.89 g and 56.02 ± 22.13 g, respectively ($P < 0.001$, Wilcoxon). Latency to withdraw from a radiant heat source fell from 11.29 ± 1.51 s (ipsilateral to the lesion) and 11.41 ± 1.98 s (contralateral) before spinal lesion to 9.40 ± 0.06 s and 9.74 ± 1.73 s, respectively, after spinal lesion ($P = 0.019$ ipsilateral to the lesion, $P = 0.014$ contralaterally, Wilcoxon; n = 11).

3.2. Motor cortex stimulation reduces hyperalgesia in animals with spinal cord injury

In our animal model of central pain, motor cortex stimulation (50 μA, 50 Hz, 300-μs square pulse, 30-minute duration, "continuous-pulse MCS") significantly reduced mechanical hyperalgesia measured immediately after the termination of MCS stimulation and every 30 minutes thereafter (Fig. 1). On the hind paw ipsilateral to spinal lesion, MCS increased mechanical thresholds from 60.00 ± 0.00 g to 92.73 ± 16.18 g and from 53.82 ± 13.75 g to 80.64 ± 20.10 g on the contralateral hind paw ($P < 0.001$ both sides, Friedman test; n = 11; Fig. 1A). Mechanical thresholds remained elevated for at least 30 minutes after the stimulation ceased and returned to prestimulation values within 60 minutes after stimulation.

Continuous-pulse MCS also significantly reduced thermal hyperalgesia. Latency to withdraw from a radiant heat source increased immediately after MCS from 7.65 ± 0.45 s to 11.12 ± 1.16 s ipsilateral to lesion and from 7.7 ± 0.72 s to 10.73 ± 1.11 s contralateral to lesion ($P < 0.05$ both sides, Friedman test; n = 6; Fig. 1B). Withdrawal latencies returned to baseline values within 30 minutes after the end of stimulation.

MCS significantly increased mechanical withdrawal thresholds and thermal withdrawal latencies in 94% (14/15) of the animals on the ipsilateral hind paw (ipsilateral to the lesion site). On the contralateral hind paw, MCS was effective in 87% (13/15) of the animals.

3.3. Reduction in hyperalgesia is dependent on stimulation parameters

Clinicians are hesitant to prescribe MCS for patients suffering from neuropathic pain because of the varied success rate and mixed outcomes of MCS treatment (see Introduction). This is further compounded by the lack of consensus on what constitutes an effective stimulation protocol and how various stimulation parameters affect the analgesia produced. Studies using repetitive

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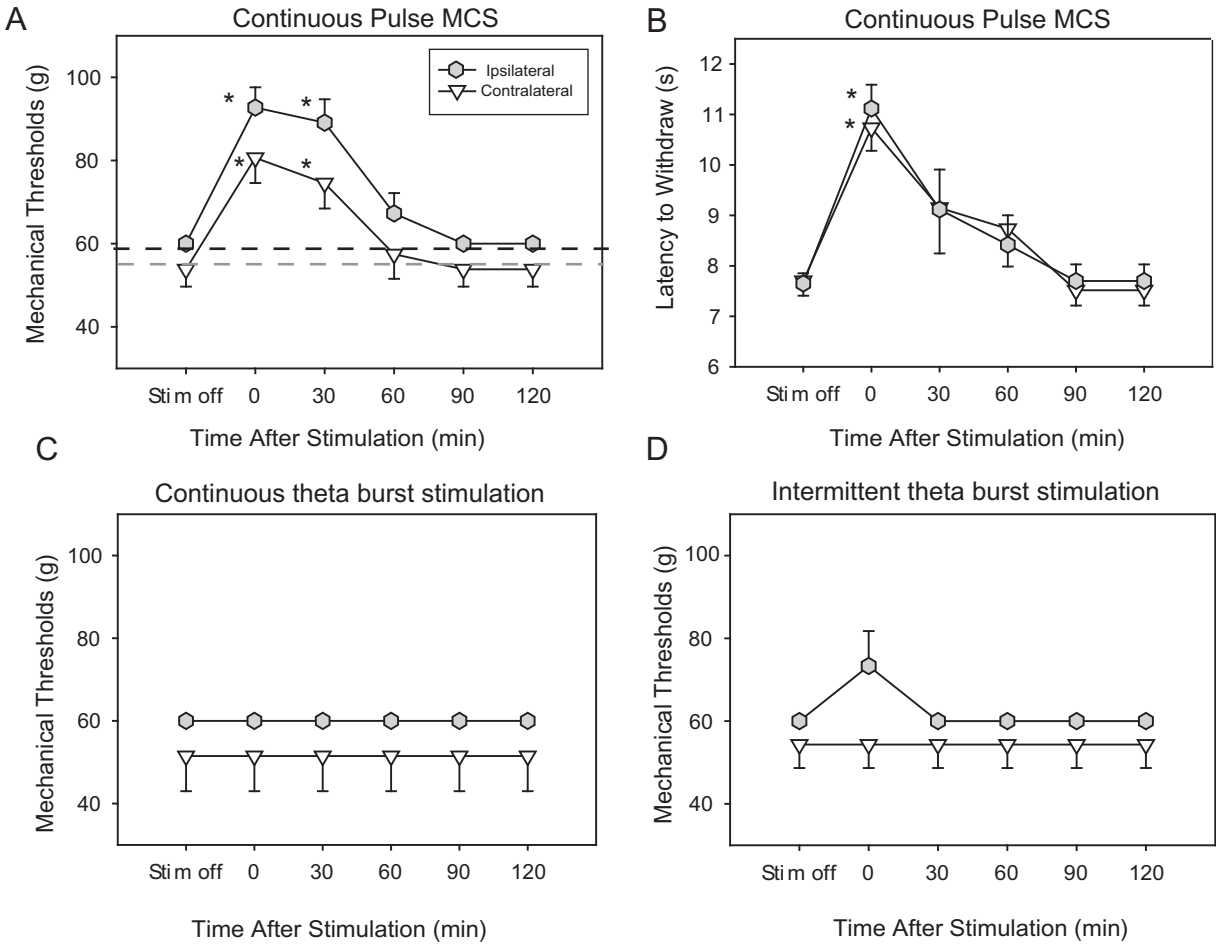

Fig. 1. Continuous-pulse motor cortex stimulation (MCS) reduced hyperalgesia. (A) Thirty minutes of continuous-pulse MCS significantly increased mechanical thresholds in both ipsilateral and contralateral hind paws (relative to the lesion) after MCS ($P < 0.001$ Friedman test followed by Dunnett's post hoc; n = 11). Stimulation off values taken immediately before stimulation, time = 0 marks the time MCS ended. Horizontal dotted lines indicate average mechanical thresholds after spinal surgery (black: hind paw ipsilateral to the lesion; gray: contralateral). (B) Continuous-pulse MCS significantly increased latency to withdraw from a radiant heat source in both hind paws immediately after 30 minutes of stimulation ($P < 0.05$ Friedman test followed by Dunnett's post hoc, n = 6). (C) Continuous theta burst stimulation did not significantly increase mechanical thresholds in either hind paw of animals with hyperalgesia (n = 6). (D) Intermittent theta burst stimulation did not significantly increase mechanical thresholds in animals with hyperalgesia (hind paw ipsilateral to the lesion: $P = 0.075$, Friedman test; n = 6). Asterisk indicates statistically significant difference ($P < 0.05$).

transcranial magnetic stimulation in healthy individuals report that theta burst stimulation (TBS) protocols can produce powerful effects on motor cortex outputs, with intermittent TBS (iTBS) being most effective [27]. Because of this, we tested whether TBS is effective in reducing hyperalgesia in our animal model of central pain.

TBS (3 stimuli at 50 Hz repeated every 200 ms [27]) had no effect on hyperalgesia measured immediately after MCS in animals with spinal cord injury (n = 6; $P = 1.0$; Friedman test; Fig. 1C). iTBS (2-second trains of TBS repeated every 10 seconds) appeared to increase mechanical withdrawal thresholds on the hind paw ipsilateral to the lesion immediately after stimulation; however, these threshold changes were not significant ($P = 0.075$, Friedman test; n = 6; Fig. 1D). No changes in withdrawal threshold were found on the contralateral side. These results indicate that changes in mechanical hyperalgesia are dependent on the stimulation protocol used and that our stimulation protocol, continuous-pulse MCS, was more effective in reducing hyperalgesia than the TBS protocols.

Next, using continuous-pulse MCS, we examined which stimulation parameters were most effective at reducing hyperalgesia in our animal model spinal cord injury pain. We varied either the intensity, the frequency, or the duration of stimulation while keeping all other parameters constant and evaluated changes in mechanical thresholds immediately after the end of MCS and at 30-minute intervals thereafter. In Fig. 2A, we show the effects of varying the intensity of stimulation on mechanical thresholds (**constant parameters**: 50 Hz, 30 minutes, 300 μs). Increasing stimulation current resulted in an intensity-dependent increase in mechanical thresholds on both the ipsilateral (ipsilateral to the spinal lesion) and contralateral hind paws. While on the ipsilateral hind paw, stimulation at the lowest intensity, 10 μA (n = 7), had no effect on the mechanical thresholds, stimulation at higher intensities did significantly increase thresholds (**prestimulation**: 60.00 ± 0.00 g; **10 μA**: 71.43 ± 19.52 g [n = 7]; **25 μA**: 82.86 ± 21.38 g [n = 7]; **50 μA**: 93.33 ± 15.57 g [n = 12]; **75 μA**: 88.57 ± 19.52 g [n = 7]; $P < 0.001$, Kruskal–Wallis followed by Dunn's post hoc; Fig 2A).

On the contralateral hind paw, however, only stimulation at 50 μA was able to significantly raise mechanical thresholds, while 10 μA, 25 μA, or 75 μA of stimulation had no significant effect on hyperalgesia (**prestimulation**: 53.82 ± 13.75 g; **10 μA:** 55.14 ± 13.23 g; **25 μA:** 55.14 ± 12.85 g; **50 μA:** 77.19 ± 24.34 g; **75 μA:** 65.71 ± 15.12 g; $P = 0.013$; Fig. 2A). Therefore, stimulation at 50 μA was most effective at reducing hyperalgesia on both hind paws.

In Fig. 2B we show the effect of varying the frequency of stimulation (**constant parameters:** 50 μA, 30 minutes, 300 μs) on mechanical hyperalgesia. Stimulating at both 50 Hz (n = 11) and 75 Hz (n = 6) resulted in a significant reduction of hyperalgesia in

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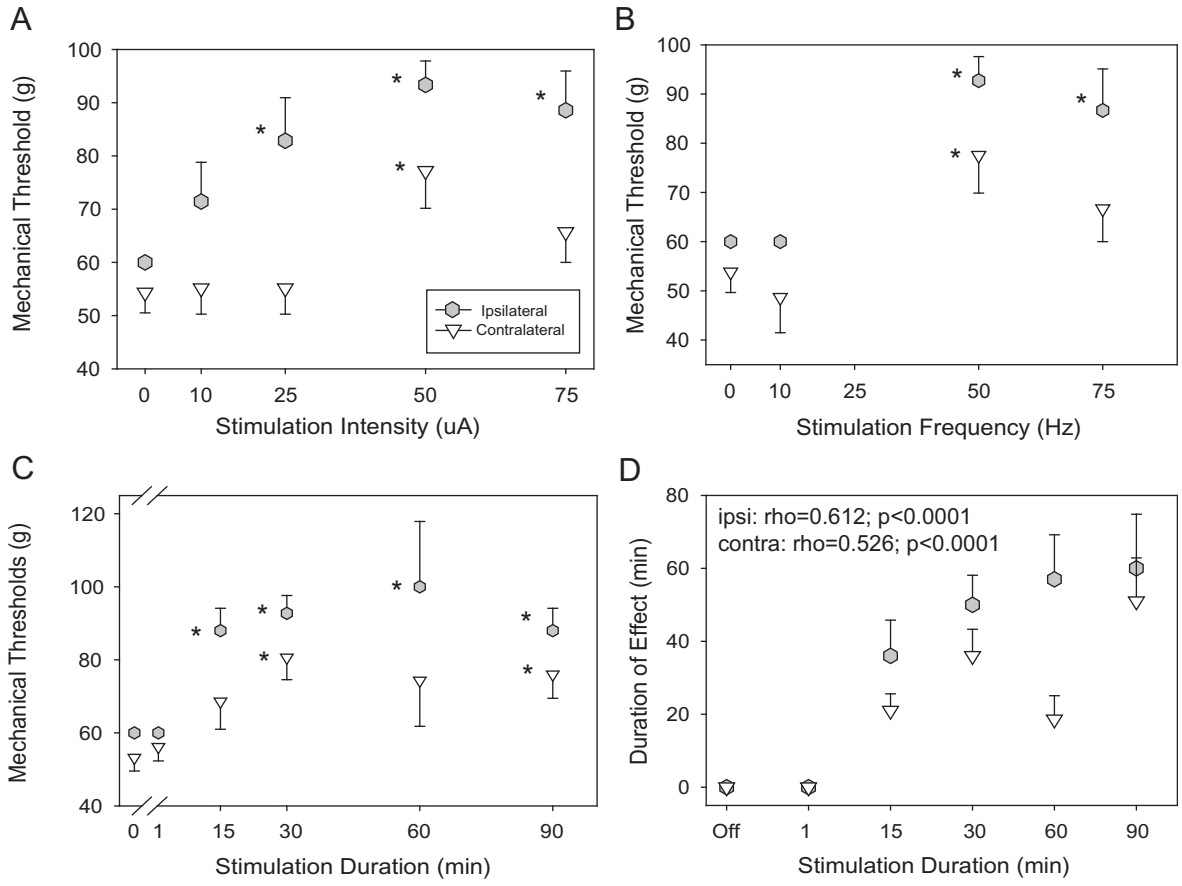

Fig. 2. Effect of motor cortex stimulation (MCS) parameters on hyperalgesia. (A) The effect of varying stimulation intensity on mechanical withdrawal thresholds. In the hind paw ipsilateral to the lesion, 25-μA (n = 7), 50-μA (n = 12), and 75-μA (n = 7) stimulation significantly increased mechanical thresholds after the end of MCS ($P < 0.001$, Kruskal–Wallis followed by Dunn's post hoc). The contralateral hind paw showed significantly increased thresholds only after 50-μA stimulation ($P = 0.013$, Kruskal–Wallis followed by Dunn's post hoc). (B) The effect of varying stimulation frequency on mechanical withdrawal thresholds. Ipsilateral thresholds (ipsilateral to the lesion) were significantly raised when M1 was stimulated at 50 Hz (n = 11) and at 75 Hz (n = 6) ($P < 0.001$, Kruskal–Wallis followed by Dunn's post hoc). Contralateral thresholds were significantly raised when MCS occurred at 50 Hz ($P = 0.024$, Kruskal–Wallis followed by Dunn's post hoc). (C) The effect of varying stimulation duration on mechanical withdrawal thresholds. Hyperalgesia in the hind paw ipsilateral to the lesion was significantly reduced after 15 minutes (n = 10), 30 minutes (n = 11), 60 minutes (n = 6), and 90 minutes (n = 10) of continuous-pulse MCS while hyperalgesia in the contralateral hind paw was significantly reduced after only 30 minutes and 90 minutes of MCS ($P < 0.001$ and $P = 0.02$, respectively, Kruskal–Wallis followed by Dunn's post hoc). (D) Duration of MCS is positively correlated with duration of post effects in both hind paws ($P < 0.001$; Spearman's).

the ipsilateral hind paw (ipsilateral to the lesion), but stimulation at 10 Hz (n = 6) had no effect (**prestimulation**: 60.00 ± 0.00 g; **10 Hz:** 60.00 ± 0.00 g; **50 Hz:** 92.73 ± 16.18 g; **75 Hz:** 86.67 ± 20.66 g; $P < 0.001$, Kruskal–Wallis followed by Dunn's post hoc; Fig. 2B).

On the hind paw contralateral to the lesion, only stimulation at 50 Hz (n = 11) was effective in reducing hyperalgesia, while neither stimulation at 10 Hz nor stimulation at 75 Hz significantly increased mechanical thresholds (**prestimulation:** 53.82 ± 13.75 g; **10 Hz:** 48.67 ± 17.56 g; **50 Hz:** 77.55 ± 25.49 g; **75 Hz:** 66.55 ± 16.33 g; $P = 0.024$, Fig. 2B). These data indicate that stimulation at 50 Hz is most effective at reducing hyperalgesia in both hind paws.

We next examined the effect of changing stimulation duration on mechanical thresholds. Over the course of 2 weeks, animals were tested using calibrated von Frey filaments immediately before and immediately after 1, 15, 30, 60, or 90 minutes of MCS (50 μA, 50 Hz, 300 μs).

In the hind paw ipsilateral to the lesion, 15 (n = 10), 30 (n = 11), 60 (n = 6), and 90 (n = 10) minutes of stimulation significantly increased mechanical thresholds after the end of MCS, but stimulation lasting 1 minute (n = 6) failed to significantly reduce hyperalgesia (**prestimulation:** 60.00 ± 0.00 g; **1 minute:** 60.00 ± 0.00 g; **15 minutes:** 88.00 ± 19.32 g; **30 minutes:** 92.73 ± 16.18 g; **60 minutes:** 100 ± 43.82 g, **90 minutes:** 88.00 ± 19.32 g; $P < 0.001$, Kruskal–Wallis followed by Dunn's post hoc; Fig. 2C).

On the contralateral hind paw (contralateral to the lesion), 30 minutes and 90 minutes of stimulation increased mechanical thresholds significantly after the end of stimulation, while stimulation lasting 1, 15, or 60 minutes failed to cause significant changes in hyperalgesia after MCS (**prestimulation:** 53.82 ± 14.08 g; **1 minute:** 56.12 ± 9.39 g; **15 minutes:** 68.3 ± 24.08 g; **30 minutes:** 80.64 ± 20.11 g; **60 minutes:** 74.33 ± 30.74 g; **90 minutes:** 76.00 ± 20.66; $P = 0.02$; Fig. 2C). Therefore, 30 minutes of stimulation was most effective at reducing hyperalgesia in both hind paws.

3.4. Reduction in hyperalgesia outlasts duration of stimulation

Human studies report that not only can MCS provide immediate relief from pain in patients, but it can also produce analgesia that lasts long after stimulation ceases (see Introduction). Therefore, we investigated the duration during which mechanical thresholds remained elevated after MCS ("post effects") in our animal model of central pain. To this end, we stimulated the motor cortex for varying durations (as described in Methods) and obtained mechanical thresholds from animals with hyperalgesia immediately after

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the end of stimulation and again at 30-minute intervals until mechanical thresholds returned to prestimulation values.

Stimulation duration positively correlated with the duration of post effects (**ipsilateral to the lesion:** $\rho = 0.61$, $P < 0.0001$; **contralateral:** $\rho = 0.526$, $P < 0.0001$, Spearman's; Fig. 2D). With increased duration, mechanical thresholds in the hind paw ipsilateral to the lesion remained elevated after the end of stimulation for the following lengths of time (see Fig. 2D; **no stimulation [n = 15]:** 0.00 ± 0.00 minutes; **1-minute stimulation:** 0.00 ± 0.00 minutes; **15-minute stimulation:** 36.00 ± 30.98 minutes; **30-minute stimulation:** 50.00 ± 31.40 minutes; **60-minute stimulation:** 57.00 ± 38.60 minutes; **90-minute stimulation:** 60.00 ± 46.90 minutes). We obtained similar results on the hind paw contralateral to the lesion (**no stimulation:** 0.00 ± 0.00 minutes; **1-minute stimulation:** 0.00 ± 0.00 minutes; **15-minute stimulation:** 21.00 ± 14.49 minutes; **30-minute stimulation:** 36.00 ± 28.23 minutes; **60-minute stimulation:** 18.60 ± 20.48 minutes; **90-minute stimulation:** 51.00 ± 37.55 minutes). Taken together, these findings suggest that the following stimulation parameters: 50 μA, 50 Hz, 300 μs for a duration of at least 15 minutes (continuous-pulse MCS), are effective at reducing hyperalgesia bilaterally in rats with spinal cord lesions.

3.5. Zona incerta stimulation mimics the effects of MCS

The data presented here demonstrate that continuous-pulse MCS significantly reduces hyperalgesia in this model of central pain. We have demonstrated previously that the development of hyperalgesia is associated with reduced activity in the inhibitory nucleus ZI in rats [33]. Because the motor cortex sends dense projections to the ventral division of ZI [35,62], we hypothesized that MCS reduces hyperalgesia by increasing activity in ZI. This hypothesis predicts that electrical stimulation of ZI will also reduce hyperalgesia.

To test this prediction we implanted bipolar stimulating electrodes in ZI of 8 animals concurrent with spinal lesion surgery (see Methods). In animals that developed hyperalgesia, we stimulated ZI and tested mechanical and thermal thresholds immediately following the termination of stimulation. Electrical stimulation in ZI (25 μA, 50 Hz, 300 μs square pulse, 15 minutes) caused a significant increase in mechanical thresholds immediately after the end of stimulation in both hind paws (**ipsilateral to the lesion:** from 63.33 ± 8.16 g to 95.55 ± 18.21 g; $P = 0.03$; **contralateral:** from 66.67 ± 10.31 g to 102.22 ± 13.12 g; $P = 0.03$, Wilcoxon; n = 6; Fig. 3A). In addition, ZI stimulation significantly increased thermal withdrawal latencies in both hind paws after the end of stimulation (**ipsilateral to the lesion:** from 6.13 ± 0.92 s to 10.05 ± 0.95 s; $P = 0.01$; **contralateral:** from 6.28 ± 0.44 s to 9.94 ± 1.28 s; $P = 0.02$, Wilcoxon; n = 8; Fig. 3B). Therefore, consistent with our hypothesis, increasing activity in ZI reduces hyperalgesia.

3.6. Inactivation of ZI prevents MCS-induced reduction in hyperalgesia

To further test our hypothesis that MCS reduces hyperalgesia by activating ZI, we investigated whether reversible inactivation of ZI occluded the effects of MCS. We implanted microdialysis cannulae in ZI as well as MCS electrodes above M1. Animals received infusions of either saline (n = 4) or 2% lidocaine into ZI during MCS and changes in mechanical withdrawal thresholds were assessed immediately after the end of stimulation and at 30-minute intervals thereafter (see Methods). Lidocaine infusion in ZI completely blocked the effects of MCS (prestimulation: 60.00 ± 0.00 g; end of stimulation: 60.00 ± 0.00 g; $P = 1.00$, Friedman test; Fig 3C). Because lidocaine inactivates sodium channels, it is possible that infusion of lidocaine inactivated fibers of passage traveling through ZI. Therefore, we repeated the experiments using γ-aminobutyric acid $(GABA)_A$ agonist muscimol (200 μM, n = 4) for more specific inactivation. Muscimol, like lidocaine, blocked MCS effects (prestimulation: 60.00 ± 0.00 g; end of stimulation: 60.00 ± 0.00 g; $P = 1.00$, Friedman). Similar results were seen in the hind paw contralateral to spinal lesion (data not shown). Importantly, infusion of the same volume of saline into ZI did not disrupt the MCS-induced reduction in hyperalgesia (prestimulation: 60.00 ± 0.00 g; end of stimulation: 100.00 ± 0 g; 30 minutes poststimulation: 84.00 ± 21.91 g; 60 minutes poststimulation: 60.00 ± 0.00 g; $P = 0.01$, Friedman test followed by Dunnett's post hoc; Fig. 3C). In all animals, we performed postmortem histological analysis to confirm correct placement of the cannula. Fig. 3D shows a small lesion in the ventral portion of ZI at the site of drug infusion, and Fig. 3E is a schematic representation of ZI and adjacent structures with reconstruction of the injection sites.

These data suggest that MCS reduces hyperalgesia by increasing activity in ZI and suggest that ZI may play an integral role in mediating the reduction in hyperalgesia observed after MCS.

4. Discussion

4.1. Reduced hyperalgesia after MCS

To date, clinical studies have failed to reveal which stimulus parameters are critical for MCS to successfully reduce hyperalgesia (see Introduction). Here, using an animal model of spinal cord injury pain, we systematically varied stimulation parameters to test the effects on hyperalgesia immediately after MCS, an advantage not available in human studies. We found that, in rats, MCS at an intensity of 50 μA and frequency of 50 Hz was most effective at reducing mechanical hyperalgesia bilaterally.

The finding that reduction of hyperalgesia after MCS extends beyond the duration of stimulation is consistent with the reported post effects in humans (see Introduction). These post effects are especially promising, as they offer a potential cure or treatment for intractable pain. Understanding the mechanisms by which MCS induces long-lasting pain relief is crucial to increasing the efficacy of MCS treatment in the clinical population.

4.2. Specificity of MCS effects

In this study, we stimulated the motor cortex but did not test the effects of stimulating other cortical structures because it has been shown that cortical stimulation in areas other than the motor cortex is not as effective in reducing pain. In humans, stimulation of the prefrontal and somatosensory cortices did not produce significant analgesia in patients with neuropathic pain [51]. Similarly, in rodents, stimulation of the primary or second somatosensory cortices or the posterior parietal cortex had little or no effect on nociceptive responses [19,29]. For these reasons, we focused our study on stimulation of the primary motor cortex.

In humans, the consensus is that MCS effects are restricted to areas contralateral to the stimulation site, and therefore stimulation is usually applied to the motor cortex contralateral to the painful region, which is normally ipsilateral to the site of the spinal cord injury. However, there are some reports that MCS resulted in analgesia on the ipsilateral side [42,58]. In addition, a recent study using repetitive transcutaneous magnetic stimulation demonstrated that MCS reduced laser-evoked pain perception bilaterally [48]. Therefore, bilateral effects cannot be ruled out. In the present study, animals developed bilateral mechanical hyperalgesia following spinal cord injury and stimulating electrodes were implanted contralateral to the lesion site. Although

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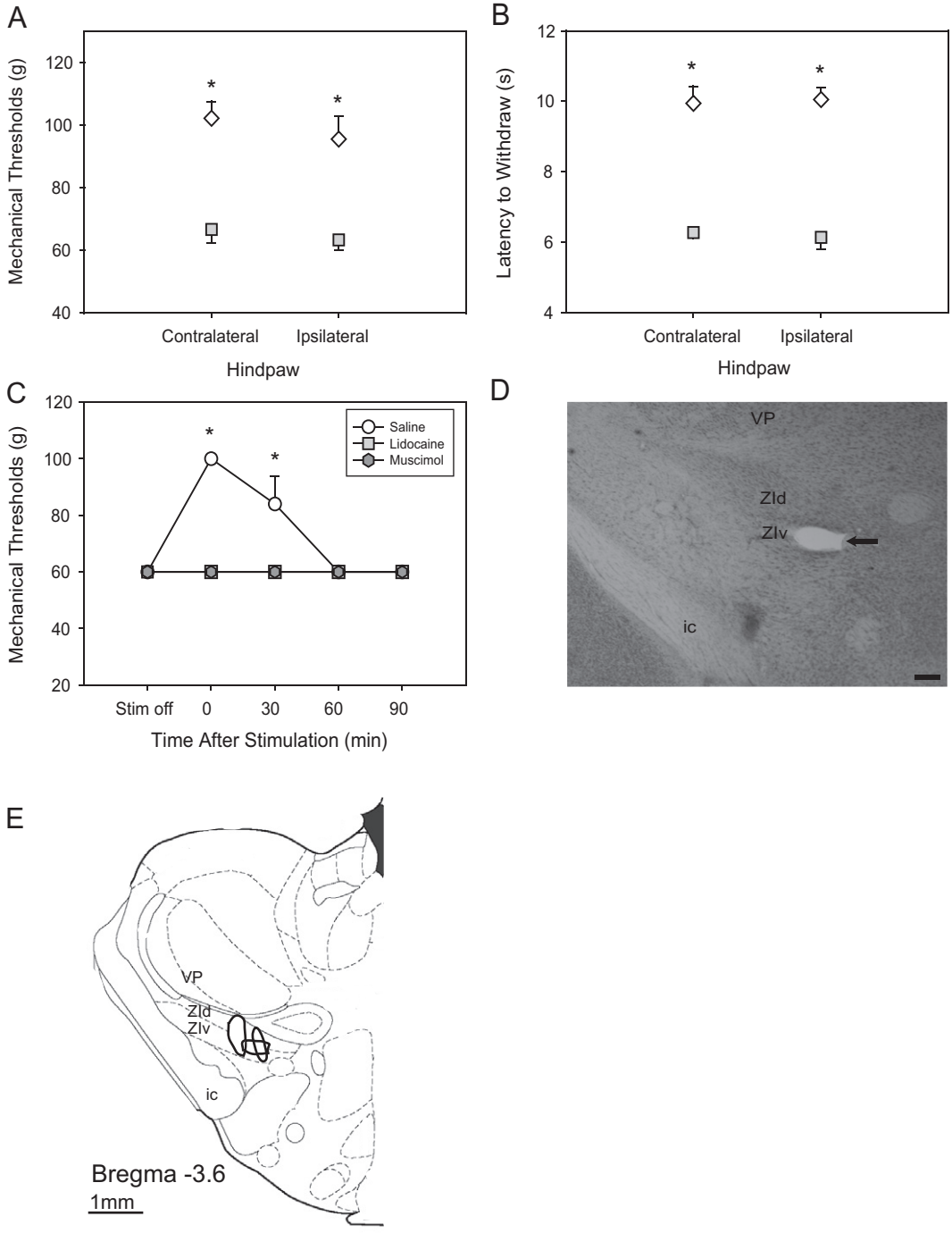

Fig. 3. Zona incerta (ZI) mediates the effects of motor cortex stimulation (MCS). ZI stimulation significantly increased (A) mechanical thresholds and (B) thermal thresholds bilaterally in animals with hyperalgesia. Square: stimulation off, diamond: stimulation on. (C) Inactivation of ZI with lidocaine or muscimol occludes the effects of MCS. The infusion of the same volume of saline had no effect on MCS-induced reduction in hyperalgesia ($P = 0.01$, Friedman test followed by Dunnett's post hoc). Ipsilateral hind paw (relative to lesion site) shown for clarity; similar effects were seen in the contralateral hind paw. (D) Representative microdialysis cannula placement in ZI. Arrow indicates small lesion produced by drug infusion, scale bar = 150 μm. (E) Schematic representation of ZI and adjacent structures and reconstruction of injection site in 3 animals. The schematic was adopted from [42] and modified. VP, ventral posterolateral thalamus; ZId, zona incerta, dorsal part; ZIv, zona incerta, ventral part; ic, internal capsule.

this makes it difficult to compare our findings to clinical situations, unilateral MCS in our animal model produced consistent bilateral reduction in mechanical withdrawal thresholds measured after the end of stimulation. The bilateral effects of MCS are consistent with previous reports in rats [66,67] and they could be due to MCS influence on structures that receive somatosensory inputs from bilateral areas of the body such as the posterior thalamus [33,47], or due to transcallosal activation of the contralateral motor cortex [3].

4.3. Animal model of central pain

In humans, a hallmark characteristic of central pain syndrome is severe, spontaneous pain [10,71]. A major limitation of animal

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models of central pain, including ours, is the inability to convincingly demonstrate that animals suffer from spontaneous pain after injury. Thus far, studies attempting to demonstrate spontaneous pain in animals have relied on behaviors such as overgrooming/autotomy, licking, guarding, and vocalization [65]. However, these metrics are not reliable, nor are they specific to pain sensations [36]. Recently, a conditioned place preference paradigm was used to demonstrate that animals with peripheral nerve injury suffer from tonic pain [28]. In the future, a strategy similar to that of the conditioned place preference paradigm may prove useful to test whether animals with spinal cord injury develop signs of spontaneous pain and to test whether MCS reduces these signs.

A potential limitation to our animal model is that damage to the spinothalamic tract results in bilateral below-level hyperalgesia. It is expected that unilateral damage to the spinothalamic tract would result in below-level pain that is restricted to areas contralateral to the injury. However, some exceptions are reported in animal and human literature. In rats, 2 different animal models demonstrate that unilateral damage to the spinal cord results in bilateral below-level thermal and mechanical hyperalgesia [12,13,64]. Additionally, in monkeys, unilateral cuts of the anterolateral quadrant of the spinal cord result in bilateral increased sensitivity to noxious stimuli [63]. Finally, in humans, below-level pain due to spinothalamic tract lesions can be bilateral [24], or even ipsilateral to the site of injury in some instances [4,68]. Although not common, these reports suggest that pain following unilateral spinal cord lesions is not always limited to a contralateral distribution [65]. Development of bilateral hyperalgesia could be due to pathologic changes in distant spinal sites affecting caudal spinothalamic tract projections, or due to changes in supraspinal targets that receive bilateral convergent inputs (eg, [33]).

4.4. Mechanisms of pain relief

Several hypotheses have been proposed to explain how MCS provides pain relief. In general, most propose that MCS enhances inhibition in one of 3 structures along the neural axis: (1) within the neocortex; (2) in the spinal cord; or (3) within the thalamus [8,44,46].

Advocates for a cortical mechanism of pain relief believe that MCS enhances activity of "non-nociceptive" sensory inputs in the primary somatosensory cortex (S1) which, in turn, inhibit nociceptive neurons in S1 that receive inputs from the spinothalamic tract [17,30]. However, this notion may be dismissed by imaging studies demonstrating that MCS was not associated with changes in cerebral blood flow in the primary motor or somatosensory cortices [21–23,44].

There are those who argue that MCS may directly or indirectly inhibit nociceptive inputs in the spinal cord. Direct inhibition is unlikely, though, because M1 does not project to the superficial layers or marginal zone of the dorsal horn [52]. An indirect role is more likely, as MCS may activate descending inhibitory systems and cause endogenous opioid release [19] (but see [66]). Despite limited support for this claim, manipulations that specifically activate endogenous opioid release, such as deep brain stimulation of the periaqueductal gray, are especially poor for the treatment of CPS [25,57].

Some authors hypothesized that MCS activates corticothalamic connections, and these in turn inhibit nociceptive processing in the thalamus [6,7,51]. In support of this hypothesis, it was argued that patients responsive to GABA or barbiturate treatment are more likely to benefit from MCS [8,9,11]. However, the specific role of the thalamus is still debatable [44], and the source of altered inhibition, the mechanisms for engagement of inhibition, and the specific nuclei affected by MCS remain to be elucidated.

Here we focus on the role of the ZI in mediating the effects of MCS. We found that reversible inactivation of ZI with lidocaine or muscimol blocks the reduction of hyperalgesia observed after MCS. In these experiments it is important to consider the time course of wearing off of lidocaine and muscimol relative to MCS post effects. A previous report [32] estimated that lidocaine effects last from 30 to 60 minutes after injection into the cortex at low concentrations (40 μg/1 μL vs 1 mg/50 μL used in this study). The same study [32] found that the effects of muscimol last from 30 to 120 minutes. Considering that the reduction in hyperalgesia after the end of MCS lasts for 30–60 minutes in control experiments (Fig. 3C) and that drug infusion continues 15 minutes into MCS (see Methods), then the effects of the drugs applied are expected to match or even outlast the post effects of MCS, especially when considering the higher concentrations of lidocaine used in our study. Future experiments using reversible inactivation while varying the concentration and timing of administration of drugs relative to stimulation may prove useful to further test the mechanisms involved in the prolonged aftereffects observed after MCS. Another possibility is that the applied drugs diffused beyond the boundaries of ZI and affected neighboring structures such as the internal capsule or the ventroposterior thalamus. These structures may be involved in mediating MCS effects and therefore, results should be interpreted with caution.

We also found that electrical stimulation of ZI mimics MCS effects and therefore, we hypothesize that MCS produces its effects by enhancing activity of ZI in rats. Enhanced activity in the GABAergic ventral division of ZI may result in increased inhibitory inputs to higher-order thalamic nuclei that are involved in nociceptive processing, specifically the posterior thalamic nucleus [33,59]. It is important to note, however, that the human thalamus is more complex than, and not completely analogous to, the rat thalamus [16]. As such, the human homologue to the rat posterior thalamic nucleus remains to be identified.

In this study, we identify the ZI as a source of inhibition that can be manipulated to produce pain relief, and describe a novel system that affects nociceptive transmission within the thalamus through corticothalamic interactions. Identifying the mechanisms involved in the short- and long-term consequences of MCS will shift current research and clinical practice paradigms and lead toward the development of molecular, pharmacologic, and physiologic methods for permanent pain relief that target these structures.

Conflict of interest statement

The authors declare no conflicts of interest related to this work.

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References

[1] Baastrup C, Finnerup NB. Pharmacological management of neuropathic pain following spinal cord injury. CNS Drugs 2008;22:455–75.
[2] Bezard E, Boraud T, Nguyen JP, Velasco F, Keravel Y, Gross C. Cortical stimulation and epileptic seizure: a study of the potential risk in primates. Neurosurgery 1999;45:346–50.
[3] Bogdanova OG, Sil'kis IG. The effects of high-frequency microstimulation of the cortex on interhemisphere synchronization in the rat motor cortex. Neurosci Behav Physiol 1999;29:515–22.
[4] Bowsher D. Contralateral mirror-image pain following anterolateral cordotomy. Pain 1988;33:63–5.

[5] Brown JA, Pilitsis JG. Motor cortex stimulation for central and neuropathic facial pain: a prospective study of 10 patients and observations of enhanced sensory and motor function during stimulation. Neurosurgery 2005;56:290–7.
[6] Canavero S, Bonicalzi V. Cortical stimulation for central pain. J Neurosurg 1995;83:1117.
[7] Canavero S, Bonicalzi V. Resolution of central pain. J Neurosurg 1999;91:715–6.
[8] Canavero S, Bonicalzi V. Extradural cortical stimulation for central pain. Acta Neurochir Suppl 2007;97:27–36.
[9] Canavero S, Bonicalzi V. Central pain syndrome: elucidation of genesis and treatment. Expert Rev Neurother 2007;7:1485–97.
[10] Canavero S, Bonicalzi V. Central pain syndrome: pathophysiology, diagnosis and management. New York: Cambridge Univ. Press; 2007.
[11] Canavero S, Pagni CA, Castellano G, Bonicalzi V, Bello M, Duca S, Podio V. The role of cortex in central pain syndromes: preliminary results of a long-term technetium-99 hexamethylpropyleneamineoxime single photon emission computed tomography study. Neurosurgery 1993;32:185–9.
[12] Christensen MD, Everhart AW, Pickelman JT, Hulsebosch CE. Mechanical and thermal allodynia in chronic central pain following spinal cord injury. Pain 1996;68:97–107.
[13] Christensen MD, Hulsebosch CE. Chronic central pain after spinal cord injury. J Neurotrauma 1997;14:517–37.
[14] Cruccu G, Aziz TZ, Garcia-Larrea L, Hansson P, Jensen TS, Lefaucheur JP, Simpson BA, Taylor RS. EFNS guidelines on neurostimulation therapy for neuropathic pain. Eur J Neurol 2007;14:952–70.
[15] Deuschl G, Herzog J, Kleiner-Fisman G, Kubo C, Lozano AM, Lyons KE, Rodriguez-Oroz MC, Tamma F, Troster AI, Vitek JL, Volkmann J, Voon V. Deep brain stimulation: postoperative issues. Mov Disord 2006;21:S219–37.
[16] Dostrovsky JO. Ascending projection systems. In: McMahon S, Koltzenburg M, editors. Wall and Melzack's textbook of pain. Oxford, UK: Churchill Livingstone; 2005. p. 187–204.
[17] Drouot X, Nguyen JP, Peschanski M, Lefaucheur JP. The antalgic efficacy of chronic motor cortex stimulation is related to sensory changes in the painful zone. Brain 2002;125:1660–4.
[18] Ebel H, Rust D, Tronnier V, Boker D, Kunze S. Chronic precentral stimulation in trigeminal neuropathic pain. Acta Neurochir (Wien) 1996;138:1300–6.
[19] Fonoff ET, Dale CS, Pagano RL, Paccola CC, Ballester G, Teixeira MJ, Giorgi R. Antinociception induced by epidural motor cortex stimulation in naive conscious rats is mediated by the opioid system. Behav Brain Res 2008;196:63–70.
[20] Fontaine D, Hamani C, Lozano A. Efficacy and safety of motor cortex stimulation for chronic neuropathic pain: critical review of the literature. J Neurosurg 2009;110:251–6.
[21] Garcia-Larrea L, Peyron R. Motor cortex stimulation for neuropathic pain: from phenomenology to mechanisms. Neuroimage 2007;37:S71–9.
[22] Garcia-Larrea L, Peyron R, Mertens P, Gregoire MC, Lavenne F, Bonnefoi F, Mauguiere F, Laurent B, Sindou M. Positron emission tomography during motor cortex stimulation for pain control. Stereotact Funct Neurosurg 1997;68:141–8.
[23] Garcia-Larrea L, Peyron R, Mertens P, Gregoire MC, Lavenne F, Le Bars D, Convers P, Mauguiere F, Sindou M, Laurent B. Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. Pain 1999;83:259–73.
[24] Graf CJ. Consideration in loss of sensory level after bilateral cervical cordotomy. Arch Neurol 1960;3:410–5.
[25] Gybels J, Kupers R. Deep brain stimulation in the treatment of chronic pain in man: where and why? Neurophysiol Clin 1990;20:389–98.
[26] Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–88.
[27] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–6.
[28] King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. Nat Neurosci 2009;12:1364–6.
[29] Kuroda R, Kawabata A, Kawao N, Umeda W, Takemura M, Shigenaga Y. Somatosensory cortex stimulation-evoked analgesia in rats: potentiation by NO synthase inhibition. Life Sci 2000;66:PL271–6.
[30] Lefaucheur JP, Drouot X, Menard-Lefaucheur I, Nguyen JP. Neuropathic pain controlled for more than a year by monthly sessions of repetitive transcranial magnetic stimulation of the motor cortex. Neurophysiol Clin 2004;34:91–5.
[31] Lima MC, Fregni F. Motor cortex stimulation for chronic pain: systematic review and meta-analysis of the literature. Neurology 2008;70:2329–37.
[32] Martin JH. Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. Neurosci Lett 1991;127:160–4.
[33] Masri R, Quiton RL, Lucas JM, Murray PD, Thompson SM, Keller A. Zona incerta: a role in central pain. J Neurophysiol 2009;102:181–91.
[34] Mersky H, Bogduk N. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. Seattle: IASP Press; 1994.
[35] Mitrofanis J, Mikuletic L. Organisation of the cortical projection to the zona incerta of the thalamus. J Comp Neurol 1999;412:173–85.
[36] Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci 2009;10:283–94.
[37] Namba S, Nakao Y, Matsumoto Y, Ohmoto T, Nishimoto A. Electrical stimulation of the posterior limb of the internal capsule for treatment of thalamic pain. Appl Neurophysiol 1984;47:137–48.
[38] Namba S, Nishimoto A. Stimulation of internal capsule, thalamic sensory nucleus (VPM) and cerebral cortex inhibited deafferentation hyperactivity provoked after gasserian ganglionectomy in cat. Acta Neurochir Suppl (Wien) 1988;42:243–7.
[39] Neafsey EJ, Bold EL, Haas G, Hurley-Glus KM, Quirk G, Sievert CF, Terreberry RR. The organization of the rat motor cortex: a microstimulation mapping study. Brain Res 1986;396:77–96.
[40] Nguyen JP, Lefaucheur JP, Decq P, Uchiyama T, Carpentier A, Fontaine D, Brugieres P, Pollin B, Feve A, Rostaing S, Cesaro P, Keravel Y. Chronic motor cortex stimulation in the treatment of central and neuropathic pain. Correlations between clinical, electrophysiological, and anatomical data. Pain 1999;82:245–54.
[41] Nguyen JP, Lefaucher JP, Le Guerinel C, Eizenbaum JF, Nakano N, Carpentier A, Brugieres P, Pollin B, Rostaing S, Keravel Y. Motor cortex stimulation in the treatment of central and neuropathic pain. Arch Med Res 2000;31:263–5.
[42] Parrent AG, Tasker RR. Can the ipsilateral hemisphere mediate pain in man? Acta Neurochir (Wien) 1992;117:89.
[43] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego, CA: Academic Press; 1998.
[44] Peyron R, Faillenot I, Mertens P, Laurent B, Garcia-Larrea L. Motor cortex stimulation in neuropathic pain. Correlations between analgesic effect and hemodynamic changes in the brain. A PET study. Neuroimage 2007;34:310–21.
[45] Peyron R, Garcia-Larrea L, Deiber MP, Cinotti L, Convers P, Sindou M, Maguiere F, Laurent B. Electrical stimulation of precentral cortical area in the treatment of central pain: electrophysiological and PET study. Pain 1995;62:275–86.
[46] Peyron R, Garcia-Larrea L, Gregoire MC, Convers P, Richard A, Lavenne F, Barral FG, Mauguiere F, Michel D, Laurent B. Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case. Pain 2000;84:77–87.
[47] Poggio GF, Mountcastle VB. A study of the functional contributions of the lemniscal and spinothalamic systems to somatic sensibility. Central nervous mechanisms in pain. Bull Johns Hopkins Hosp 1960;106:266–316.
[48] Poreisz C, Csifcsak G, Antal A, Levold M, Hillers F, Paulus W. Theta burst stimulation of the motor cortex reduces laser-evoked pain perception. Neuroreport 2008;19:193–6.
[49] Quiton RL, Masri R, Thompson SM, Keller A. Abnormal activity of primary somatosensory cortex in central pain syndrome. J Neurophysiol 2010;104:1717–25.
[50] Ren K. An improved method for assessing mechanical allodynia in the rat. Physiol Behav 1999;67:711–6.
[51] Saitoh Y, Shibata M, Hirano S, Hirata M, Mashimo T, Yoshimine T. Motor cortex stimulation for central and peripheral deafferentation pain. Report of eight cases. J Neurosurg 2000;92:150–5.
[52] Schoenen J, Grant G. Spinal cord: connections. In: Paxinos G, Mai JK, editors. The human nervous system. Amsterdam: Elsevier Academic Press; 2004. p. 233–50.
[53] Sol JC, Casaux J, Roux FE, Lotterie JA, Bousquet P, Verdie JC, Mascott C, Lazorthes Y. Chronic motor cortex stimulation for phantom limb pain: correlations between pain relief and functional imaging studies. Stereotact Funct Neurosurg 2001;77:172–6.
[54] Son BC, Lee SW, Choi ES, Sung JH, Hong JT. Motor cortex stimulation for central pain following a traumatic brain injury. Pain 2006;123:210–6.
[55] Son UC, Kim MC, Moon DE, Kang JK. Motor cortex stimulation in a patient with intractable complex regional pain syndrome type II with hemibody involvement. Case report. J Neurosurg 2003;98:175–9.
[56] Tani N, Saitoh Y, Hirata M, Kato A, Yoshimine T. Bilateral cortical stimulation for deafferentation pain after spinal cord injury. Case report. J Neurosurg 2004;101:687–9.
[57] Tasker RR. Microelectrode findings in the thalamus in chronic pain and other conditions. Stereotact Funct Neurosurg 2001;77:166–8.
[58] Tasker RR, Parrent AG, Kiss ZHT, Davis K, Dostrovsky JO. Surgical treatment of stroke-induced pain. Stereotact Funct Neurosurg 1994;62:311–2.
[59] Trageser JC, Keller A. Reducing the uncertainty: gating of peripheral inputs by zona incerta. J Neurosci 2004;24:8911–5.
[60] Tsubokawa T, Katayama Y, Yamamoto T, Hirayama T, Koyama S. Treatment of thalamic pain by chronic motor cortex stimulation. Pacing Clin Electrophysiol 1991;14:131–4.
[61] Tsubokawa T, Katayama Y, Yamamoto T, Hirayama T, Koyama S. Chronic motor cortex stimulation in patients with thalamic pain. J Neurosurg 1993;78:393–401.
[62] Urbain N, Deschenes M. Motor cortex gates vibrissal responses in a thalamocortical projection pathway. Neuron 2007;56:714–25.
[63] Vierck CJJ, Greenspan JD, Ritz LA. Long-term changes in purposive and reflexive responses to nociceptive stimulation following anterolateral chordotomy. J Neurosci 1990;10:2077–95.
[64] Vierck CJJ, Light A. Effects of combined hemotoxic and anterolateral spinal lesion on nociceptive sensitivity. Pain 1999;83:447–57.
[65] Vierck CJJ, Siddall P, Yezierski RP. Pain following spinal cord injury: animal models and mechanistic studies. Pain 2000;89:1–5.

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[66] Viisanen H, Pertovaara A. Antinociception by motor cortex stimulation in the neuropathic rat: does the locus coeruleus play a role? Exp Brain Res 2009;201:283–96.
[67] Viisanen H, Pertovaara A. Roles of the rostroventromedial medulla and the spinal 5-HT(1A) receptor in descending antinociception induced by motor cortex stimulation in the neuropathic rat. Neurosci Lett 2010;476:133–7.
[68] Walker AE. The spinothalamic tract in man. Arch Neurol Psychiatr 1940;43:284–98.
[69] Wang G, Thompson SM. Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. J Neurosci 2008;28:11959–69.
[70] Weiss DS, Keller A. Specific patterns of intrinsic connections between representation zones in the rat motor cortex. Cerebral Cortex 1994;4:205–14.
[71] Yezierski RP. Pain following spinal cord injury: pathophysiology and central mechanisms. Prog Brain Res 2000;129:429–49.

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